Syntrophic co-cultures of *Clostridium* organisms to produce higher alcohols and other C6-C8 metabolites

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Project Goals: To develop syntrophic *Clostridium* co-culture systems for producing intermediate carbon-chain length metabolites (C4-C8) and their derivatives that can be used as chemicals or serve as biofuels and their precursors.

Clostridium organisms are of major importance for developing new technologies to produce biofuels and chemicals. Three major types of *Clostridium* organisms have been the focus of studies for the sustainable production of fuels and chemicals. Solventogenic clostridia are capable of utilizing a large variety of biomass-derived carbohydrates such as hexoses, pentoses, disaccharides, and hemicellulose, and can produce a good number of C2-C4 chemicals. Acetogenic clostridia can fix inorganic H_2, CO_2, and CO to generate C2 acids and alcohols. Other specialized clostridia possess diverse biosynthetic capabilities for production of a wide variety of metabolites including C4 – C8 carboxylic acids and alcohols, which could serve as commodity chemicals, biofuels, or biofuel precursors. The majority of previous work with clostridia focused on optimizing single-organisms systems for production of biochemical. In nature, microorganisms live in complex communities where syntrophic interactions result in superior resource utilization. Here, we first examined a synthetic syntrophy consisting of the solventogen *Clostridium acetobutylicum*, which converts simple and complex carbohydrates into a variety of chemicals, and the acetogen *C. ljungdahlii*, which fixes CO_2. This synthetic co-culture achieved carbon recoveries into C2-C4 alcohols almost to the limit of substrate-electron availability, with minimal H_2 and CO_2 release. The syntrophic co-culture produced robust metabolic outcomes over a broad range of starting population ratios of the two organisms. Finally, the co-culture exhibited unique direct cell-to-cell interactions and material exchange among the two microbes, which enabled unforeseen rearrangements in the metabolism of the individual species that resulted in the production of non-native metabolites, namely isopropanol and 2,3-butanediol. Next, to expand this co-culture system to include *C. kluyveri*, which can metabolite ethanol and acetate to produce C6 and C8 carboxylic acids. Both *C. acetobutylicum* and *C. ljungdahlii* produce ethanol and acetate, which makes *C. kluyveri* and ideal partner for a triple synthetic co-culture system capable to converting biomass-derived carbohydrates to C6 and C8 biochemicals.

^{13C}-based Metabolic Flux Analysis (MFA) will be used to gain insight into the regulation of cell growth and product formation pathways, and to identify metabolic bottlenecks. Currently, use of stable-isotope (e.g. ^{13}C) tracers combined with measurements of isotopic labeling by mass spectrometry represents the state-of-the-art in flux determination. After intense research and

development in past two decades, ^{13}C-based MFA methods are now widely used to probe fluxes in microbes. Metabolic fluxes will be studied using ^{13}C MFA in *C. kluyveri*, *C. acetobutylicum*, and *C. ljungdahlii* under mono- and co-culture conditions to identify key changes in metabolism of each organism.

To predict and better understand the co-culture interactions and predict steady-state organism abundances, a consortium model consisting of *C. kluyveri*, *C. acetobutylicum*, and *C. ljungdahlii* will be constructed using the SteadyCom framework. This will be done by standardizing the biomass equations and metabolite naming conventions for existing genome-scale models (GSMs), and updating each GSM using RNAseq and ^{13}C-fluxomics procured under varying experimental conditions. ^{13}C-fluxomics and RNAseq data will be used to infer regulatory events in each organism to simulate and compare transient monoculture and co-culture population dynamics. Computational strain design algorithms, such as OptKnock, OptForce, will be then customized for consortium models and combined with the SteadyCom framework to identify genetic perturbation targets in the clostridia consortium that lead to the overproduction of C6 and C8 biochemicals.

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