Phylogenomics of Solvent-Producing *Clostridium* Species To Enable Carbon-Negative Production of Acetone and Isopropanol

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Project Goals: Non-model organisms have unique traits and offer significant advantages and benefits for biomanufacturing. One example is gas fermenting acetogens capable of converting low-cost waste feedstocks to fuels and chemicals, deployed today at commercial scale for conversion of steel mill emissions to ethanol. Yet, engineering these non-model organisms is challenging due to lower transformation and recombination efficiencies, longer cycle times and a more limited set of genetic tools compared to model organisms *E. coli* or yeast. Cell-free systems can guide and accelerate non-model organism strain development. We are establishing a new interdisciplinary venture, the clostridia Foundry for Biosystems Design (cBioFAB) that combines advancements in cell-free and *Clostridium* engineering metabolic engineering to develop industrial-robust production strains for conversion of lignocellulosic biomass to next-generation biofuels and bioproducts.

Abstract: We recently described optimized gas-fermenting *Clostridium autoethanogenum* strains for continuous at scale production of acetone or isopropanol from syngas at rates of up to ~3 g/L/h with ~90% selectivity. Life cycle analysis (LCA) confirmed the process is carbon negative and offers >160% greenhouse gas (GHG) savings over current production routes[1]. The multi-disciplinary approach involved screening a historical industrial strain collection for superior enzymes, omics analyses, kinetic modelling, cell-free prototyping to optimize flux, and fermentation scale-up to an industrial pilot. Gas fermentation using carbon-fixing microorganisms offers an economically viable and scalable solution with unique feedstock and product flexibility that has been commercialized recently[2].

The mixed acetone-butanol-ethanol (ABE) fermentation was one of the first industrial fermentation processes for chemicals production and its peak global production reached >500 metric tons per annum. The largest and most comprehensive strain collection of solvent-producing clostridia was assembled by Prof. David Jones (University of Otago, New Zealand), which form the basis of this genome resource project. Genome sequences for this collection were generated

and determined to facilitate biosystems design for biofuel and chemical production and provide insights into bacteriophage infection and adaptive immunity via CRISPR-Cas and other systems.

We describe the genomes for seven *C. acetobutylicum*, 194 *C. beijerenckii*, five *C. butyricum*, 57 *C. saccharobutylicum*, four *C. saccharoperbutylacetonicum* three *C. tetanomorphum* and a new candidate species, which were classified using genome-wide average nucleotide identities comparisons and phylogenetic tree analysis. For each species the number of new genomes has been increased significantly and this study increases number of core and accessory protein families, with ~19% more protein families overall and a concomitant increase in phylogenetic diversity across the genus. We describe 221 amino acid sequences for core acetone-butanol-ethanol (ABE) genes that were unique within the collection and new sequences not previously in public databases. We generated a combinatorial library of the acetone-forming genes thiolase, acetoacetate CoA-transferase and acetoacetate decarboxylase that we randomly screened for increased production in gas-fermenting *C. autoethanogenum* and were able to find a set that led to a 22-fold improved performance over designs with the reference genes. Along with acetone, we observed production of unwanted side products such as 3-hydroxybutyrate due to interaction of the heterologous acetone pathway and native *C. autoethanogenum* metabolism. We retrofitted a cell-free prototyping system we had previously developed (iPROBE)[3] to identify respective candidate genes which were subsequently deleted.

To facilitate future genome editing, we further mined the collection for new CRISPR systems. CRISPR arrays were unevenly distributed and were broadly classified into 2 types: a complete Type I-B CRISPR-Cas system, and a partial Type I-B CRISPR-Cas system, which lacks genes associated with CRISPR spacer integration. An analysis of CRISPR array sequences supports phages infecting industrial clostridia described here are mostly distinct from previously isolated *Clostridium* phages. Spacer hits indicate that CRISPR-based phage defense was mostly used against phages infecting other members of the same species, and rarely against phages infecting other related species of clostridia. Cellulosomal elements were retrieved only from *C.acetobutylicum* and *C. saccharoperbutylacetonicum*, as expected. The 271 high-quality genome sequences for solvent producing clostridia are a resource[4] that will enable synthetic biology and strain development for a range of production systems.

References:

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