13. Metabolic Engineering of *Clostridium Thermocellum* for High-Yield Ethanol Production

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**Project Goals:** The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC’s approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Clostridium thermocellum is a leading candidate organism for implementing a CBP strategy for biofuel production due to its native ability to rapidly consume cellulose and its existing ethanol production pathway. C. thermocellum converts cellulose and soluble celloexodehierins such as cellobiose to lactate, formate, acetate, H2, ethanol, amino acids, and other products. A mutant strain of C. thermocellum was constructed to remove major side product formation, resulting in C. thermocellum ΔhydG Δldh Δpfl Δpta-ack. This strain no longer produced formate, acetate and lactate; hydrogen production decreased by four fold; and the ethanol yield doubled compared with the wild type on cellobiose, crystalline cellulose Avicel, and pretreated biomass. As Avicel loadings increased from 5 g/L to 50 g/L in batch serum bottles, product titers did not increase in the wild-type C. thermocellum beyond that achieved in 5 g/L Avicel, presumably limited by the drop in pH associated with production of organic acids. C. thermocellum ΔhydG Δldh Δpfl Δpta-ack, on the other hand, continued to show increased ethanol titers to a loading of 20 g/L Avicel. While this mutant exhibited a higher ethanol yield, the growth rate was negatively impacted. Therefore, faster growing mutants were enriched by serial transfer. After 1500 generations, individual evolved strains were isolated from the population via single colony purification and were resequenced.

Of the evolved mutants, all produced ethanol at higher yield than the parent C. thermocellum ΔhydG Δldh Δpfl Δpta-ack, including one that produced ethanol at >80% of theoretical yield. Correlations between genotypes and phenotypes for these strains will be discussed.

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