

## Using Multi-Omic Data to Understand the Response of *Clostridium thermocellum* to Deletions of Genes for Lactate and Acetate Production

Evert Holwerda<sup>1,3</sup>, Daniel G. Olson<sup>1,3\*</sup> ([daniel.g.olson@dartmouth.edu](mailto:daniel.g.olson@dartmouth.edu)), David Stevenson<sup>2,3</sup>, Daniel Amador-Noguez<sup>2,3</sup>, Lee R. Lynd<sup>1,2</sup> and Gerald A. Tuskan<sup>3</sup>

<sup>1</sup>Dartmouth College, Hanover; <sup>2</sup>The University of Wisconsin, Madison; <sup>3</sup>The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee

<http://cbi.ornl.gov>

**Project Goals:** The Center for Bioenergy Innovation (CBI) vision is to *accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain*. CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

*Clostridium thermocellum* is a promising candidate for the conversion of lignocellulosic feedstocks to biofuels due to its native ability to solubilize cellulose. Initial metabolic engineering efforts focused on improving ethanol production by deleting competing pathways for carbon flux (i.e., lactate and acetate production). Strains of *C. thermocellum* with deletions of both the lactate production pathway (*ldh* gene) and acetate production pathway (*pta* and *ack* genes) were constructed (A1 lineage). Subsequently, another set of strains was constructed with similar deletions (A2 lineage). Initially, ethanol production remained unchanged in both lineages. After a short period of adaptation, ethanol production improved in some strains, but not others. In these strains, carbon flux was redirected to pyruvate and amino acid production. As a means of understand the different fate of these parallel engineering strategies, we grew both sets of strains in chemostats. We identified three clusters of fermentation phenotypes. The first cluster is the wild-type phenotype, where cells produce a 1:1 ratio of ethanol and acetate. Strains with deletions of *hpt*, *spo0A* and *ldh* are in the first cluster. The second cluster phenotype involves production of ethanol, pyruvate and valine (i.e., flux was diverted from acetate to pyruvate and valine). Introducing the *pta* deletion to a strain in cluster 1 was sufficient to move it to cluster 2. The third cluster includes strains that produce mainly ethanol (about 50% of the maximum theoretical yield). Adaptation by serial transfer was sufficient to move strains from cluster 2 to cluster 3, at least in some cases. The resulting strains were resequenced and gene expression was analyzed by RNAseq. A mutation that causes a D494G mutation in the bifunctional alcohol and aldehyde dehydrogenase protein (AdhE) was observed to correlate with increased ethanol production (the change from cluster 2 to cluster 3 fermentation phenotype). To test the effect of this mutation, it was re-introduced into a strain without *ldh* or *pta* mutations and found to

reproduce about 90% of the increase in ethanol production. Ethanol production in *C. thermocellum* is limited by NADH availability rather than acetyl-CoA availability. Introducing a point mutation into the AdhE protein that changed its cofactor specificity allowed NADPH to be used for ethanol production, which increased flux through this pathway. There is no evidence that ethanol production in *C. thermocellum* is regulated at the transcriptional level. Information learned here will facilitate our understanding of our C4 alcohol and C6 ester fuels targets.

*The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science*