201. Elimination of Hydrogenase Post-translational Modification in *Clostridium thermocellum* Blocks H_2 Production and Increases Ethanol Yield

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESCS'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 for converting plant biomass into biofuels via consolidated bioprocessing. 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 consolidated bioprocessing (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 cellobiose to lactate, formate, acetate, H_2, ethanol, amino acids, and other products. Elimination of the pathways leading to products such as H_2 could redirect carbon flux towards ethanol production. Rather than delete each hydrogenase individually, we targeted a hydrogenase maturase gene (*hydG*), which is involved in converting the three [FeFe] hydrogenase apoenzymes into holoenzymes by assembling the active site. This functionally inactivated all three [FeFe] hydrogenases simultaneously, as they were unable to make active enzymes. In the Δ*hydG* mutant, the [NiFe] hydrogenase-encoding *ech* was also deleted to obtain a mutant that functionally lacks all hydrogenase. The ethanol yield increased nearly 2-fold in Δ*hydG*Δ*ech* compared to wild type, and H_2 production was below the detection limit. Interestingly, Δ*hydG* and Δ*hydG*Δ*ech* exhibited improved growth in the presence of acetate in the medium.

Transcriptomic and proteomic analysis reveal that genes related to sulfate transport and metabolism were up-regulated in the presence of added acetate in Δ*hydG*, resulting in altered sulfur metabolism. Further genomic analysis of this strain revealed a mutation in the bi- functional alcohol/aldehyde dehydrogenase *adhE* gene, resulting in a strain with both NADH- and NADPH-dependent alcohol dehydrogenase activities, whereas the wild-type strain can only utilize NADH. This is the exact same *adhE* mutation found in ethanol tolerant *C. thermocellum* strain E50C, but Δ*hydG*Δ*ech* is not more tolerant to ethanol than the wild-type. Targeting protein post-translational modification represents a promising new approach to target multiple enzymes simultaneously for metabolic engineering.

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