198. The D494G Point Mutation in the Bifunctional Alcohol and Aldehyde Dehydrogenase of *Clostridium thermocellum* Leads to Improved Ethanol Production

Dan Olson[1,2*] (dan268@gmail.com), Tianyong Zheng,[1,2] Adam Guss,[2] Daniel Amador-Noguez,[3] Lee Lynd[1,2] and **Paul Gilna[2] (BESC PI)**

[1]Dartmouth College, Thayer School of Engineering, Hanover, New Hampshire; [2]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [3]University of Wisconsin, Madison

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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). 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.

Previously we have engineered *Clostridium thermocellum* for increased ethanol production by eliminating lactate and acetate production. The resulting strain did not grow well and carbon flux was diverted to amino acid production instead of ethanol. The strain was evolved by serial transfer and ethanol production increased. To understand the reason for this change, the strain was resequenced and compared to the unevolved strain. A SNP in the bifunctional alcohol/aldehyde dehydrogenase (adhE) was found that creates a D494G substitution in the amino acid sequence. The mutation appears to alter the cofactor specificity for the alcohol dehydrogenase reaction and may explain the reason for the additional ethanol production.

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