

Pyruvate Decarboxylase: Rationally Evolving Thermostable Enzymes for Metabolic Engineering

Deanne W. Sammond^{1,2*} (Deanne.Sammond@nrel.gov), Daehwan Chung,^{1,2} Bryon Donohoe,^{1,2} Markus Alahuhta,^{1,2} Nicholas S. Sarai,¹ Michael E Himmel,¹ Vladimir Lunin,^{1,2} Adam M Guss,² Yannick J Bomble,^{1,2} and **Paul Gilna**²

¹National Renewable Energy Laboratory, Golden, Colorado; ²BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee

<http://bioenergycenter.org>

Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus*) to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Strategies for the biological production of chemicals have focused on metabolically engineering organisms to produce target chemicals near theoretical yields. Streamlining metabolic pathways by eliminating branched points is a common approach to increase chemical yields, generally achieved by removing native metabolic enzymes. Alternatively, introducing a non-native enzyme can provide a metabolic “short cut,” bypassing branched points. Here we aim to introduce the mesophilic enzyme, pyruvate decarboxylase (PDC) into a thermophilic organism to introduce just such a metabolic short cut in order to increase the production of ethanol. We have developed an *in silico-in vitro-in vivo* platform to rationally evolve the mesophilic PDC for enhanced thermostability. Importantly we find that cooperative effects for the thermostabilizing mutations are critical in our design of an enzyme with a significantly enhanced lifetime at elevated temperatures.

PDC converts pyruvate to acetaldehyde through a non-oxidative decarboxylation mechanism, a reaction that is part of the fermentative process under anaerobic conditions. PDC is found in mesophilic organisms, yet to date has not been identified in thermophiles.¹ The metabolic routes in fermentative thermophilic organisms from pyruvate to ethanol are branched and produce a number of additional products including acetic acid, formic acid and lactic acid. Expression of PDC in a fermentative thermophile would bypass metabolic branched points, channeling more pyruvate directly to acetaldehyde for conversion to ethanol. Additionally, PDC uses a non-oxidative

mechanism and therefore will not consume the limited supply of redox cofactors such as NADH, NADPH, or ferredoxin.

PDC is a 260 kDa homotetrameric enzyme with two active sites buried at each dimer interface. A thermostable PDC must include stabilization of the monomer to resist denaturation at high temperatures and stabilization of the interactions between the individual units to maintain the active complex. PDC from the mesophile *Zymonomas mobilis* has an optimum growth temperature of 30°C, while the optimum growth temperature of the host, *Caldicellulosiruptor hydrothermalis*, is 65°C.² Our aim is to identify stabilizing mutations located in the different regions of PDC, defining these regions as specific sub-domains including the dimer and tetramer interfaces, the surface and the core of the protein. Multiple protein design approaches are required to identify mutations located in each of these regions. We utilize the protein design software, Rosetta, for our *in silico* mutagenesis.^{3,4} Rosetta offers the benefit of having many distinct algorithms designed to identify thermostabilizing mutations, allowing us to target the various regions of the PDC.

References

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