Metabolic Network Modeling of Clostridium thermocellum for Systems Biology and Metabolic Engineering

R. Adam Thompson, Sergio Garcia, Sanjeev Dahal, Intawat Nookaew, Donovan S. Layton, Adam M. Guss, Dan G. Olson, Lee R. Lynd, Cong T. Trinh (ctrinh@utk.edu), and Paul Gilna

1University of Tennessee, Knoxville; 2BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; 3Bredesen Center for Interdisciplinary Research and Education, Knoxville, Tennessee; 4Thayer School of Engineering, Dartmouth College, Hanover, New Hampshire

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.

Clostridium thermocellum is a gram-positive thermophile that can directly convert lignocellulosic material into commercially relevant chemicals such as biofuels and biochemicals. Its metabolism contains many branches and redundancies, which limit the production of biofuels and biochemicals at industrially relevant yields and titers. In order to guide the experimental efforts required to overcome these barriers, we built two models of C. thermocellum metabolism. Through an extensive literature review, we first constructed a model of the core metabolism of C. thermocellum. This model was experimentally validated and served to investigate the range of phenotypes of C. thermocellum in response to significant perturbation of energy and redox pathways. The results revealed a complex, robust redox metabolism of C. thermocellum. By incorporating experimental data into this core model, we identified redox bottlenecks hindering high-yield ethanol production in C. thermocellum.1 With the recently published sequence of a genetically-tractable strain C. thermocellum DSM 1313, the KEGG database as a scaffold, and further literature review, we expanded the core model into a genome scale model (iAT601).2 This model constitutes a knowledge base for the organism, including detailed metabolic information, as well as gene protein reaction association. These features allow us to conduct studies on the impact of secondary metabolisms, isozymes, media composition, and provide a more solid basis for computational strain design. We used several sets of experimental data to train the model, e.g., estimation of the adenosine
triphosphate (ATP) requirement for growth-associated maintenance (13.5 mmol ATP/g DCW/hr) and cellulosome synthesis (57 mmol ATP/g cellulosome/hr). Using our tuned model, we (i) predicted the experimentally observed differences in cell biomass yield based on which cellodextrin species is assimilated, (ii) analyzed the experimentally quantified differences in fermentation profiles (i.e., the ethanol to acetate ratio) between cellobiose- and cellulose-grown cultures, for which we inferred potential regulatory mechanisms to explain the phenotypic differences, (iii) elucidated growth cessation and overflow metabolism in *C. thermocellum* DSM1313 at high cellulose loading, and (iv) designed over 250 genetic modification strategies with the potential to optimize ethanol production, 6,155 for hydrogen production, and 28 for isobutanol production. Our developed genome-scale model iAT60I can serve as a high-quality platform for accurately predicting complex cellular phenotypes under a variety of conditions as well as model-guided rapid strain engineering to produce industrial biofuels and chemicals of interest.

**References**


*The BioEnergy Science Center 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.*