Systems Biology-Based Optimization of Extremely Thermophilic Lignocellulose Conversion to Bioproducts

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Project Goals: We are using systems biology-guided approaches to develop a non-model, microbial metabolic engineering platform based on the most thermophilic lignocellulose-degrading organism known, *Caldicellulosiruptor bescii* (T_{opt} 78°C). This work leverages recent breakthrough improvements in the molecular genetic tools for *C. bescii*, complemented by a comprehensive understanding of its metabolism and physiology gained over the past decade of study in the PIs' laboratories. We are applying the latest metabolic reconstruction and modeling approaches to optimize biomass to product conversion using switchgrass as a model plant, and acetone and other industrial chemicals as targets. The over-arching goal is to demonstrate that a non-model microorganism, specifically an extreme thermophile, can be a strategic metabolic engineering platform for industrial biotechnology using a systems biology-based approach.

Bio-processing above 70°C can have important advantages over near-ambient operations. Highly genetically modified microorganisms typically have a fitness disadvantage and can be easily overtaken in culture by contaminating microbes. The high growth temperature of extreme thermophiles precludes growth or survival of virtually any contaminating organism or phage. This reduces operating costs associated with reactor sterilization and maintaining a sterile facility. In addition, at industrial scales, heat production from microbial metabolic activity vastly outweighs heat loss through bioreactor walls such that cooling can be required. Extreme thermophiles have the advantage that non-refrigerated cooling water can be used, and heating requirements can be met with low-grade steam, typically in excess capacity on plant sites. This project is leveraging recent developments in the PIs' labs for *C. bescii* that enable the proposed effort (1-11). We are developing approaches that provide a comprehensive description of this bacterium's physiology and metabolism to inform metabolic engineering strategies, validate the models with experimental data, and demonstrate that unpretreated lignocellulose can be converted into value-added industrial chemicals at bioreactor scale. The specific aims of this research are: 1) to construct and test a robust metabolic model based on a metabolic reconstruction of *C. bescii* growing on the simple sugars, glucose and xylose, 2) to construct and test a robust metabolic model of *C. bescii* growing on complex biomass-related sugars, cellulose and xylan, 3) to optimize the production of acetone and other industrial chemicals from simple sugars guided by metabolic modeling, and 4) to demonstrate conversion of cellulose, xylan, cellulose/xylan, and the model biomass switchgrass to valuable fermentation products.

At present, high temperature chemostat cultures are being used in conjunction with transcriptomic analysis to determine bioenergetic parameters and gene regulation patterns for *C. bescii* growth on lignocellulose-relevant sugars, including glucose, xylose, cellulose, and xylan. Notably, an alternative glycolytic pathway was recently identified in *C. bescii*, and the role of this pathway in regulating redox pools and electron flux is currently under investigation (12). Additionally, a strain of *C. bescii* has now been engineered to produce acetone during growth on cellobiose. Furthermore, vectors for engineering strains to produce other industrial chemicals have been constructed. These efforts are informing comprehensive metabolic reconstruction and modeling analyses with the ultimate goal of optimizing the production of useful products from renewable feedstocks by recombinant strains of *C. bescii*.

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This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019391