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.

Bioprocessing above 70°C has important advantages over near-ambient operations. These include resistance to contaminating organisms or phages, lower utility costs by using low-grade steam for heating and non-refrigerated water for cooling and reduced operating costs for maintenance of reactor and facility sterility. Additionally, generation of volatile products can reduce downstream separation costs, which typically account for a significant portion of operating costs. To develop *C. bescii* into a bioprocessing platform for conversion of unpretreated lignocellulose into industrially relevant chemicals, a comprehensive metabolic and regulatory reconstruction is necessary to provide a detailed description of this bacterium's physiology and metabolism and, more importantly, inform metabolic engineering strategies (1). This project leverages recent developments in *C. bescii* biomass deconstruction, genetic tools and strains and characterization of an alternate glycolytic pathway to experimentally validate ongoing modeling efforts (2,4-7). Recent metabolic engineering efforts in *C. bescii* demonstrated the ability to upgrade organic acids to alcohols through an aldehyde ferredoxin oxidoreductase and alcohol dehydrogenase (AOR-adhA pathway) (3) and the production of acetone from cellulose (8). Additional *C. bescii* strains are currently being constructed to study energy carrier usage and carbon flow and produce industrially relevant chemicals. High temperature continuous cultures and transcriptomic analyses are being employed to determine bioenergetic parameters and gene regulation patterns for growth of *C. bescii* strains. Additionally, fermentation profiling will be performed with lignocellulose-relevant sugars, including glucose, xylose, and cellobiose. These data will be used to refine the metabolic reconstruction and modeling analyses to inform metabolic engineering strategies that improve carbon and energy flow toward chemical production pathways, with the goal of demonstrating bioreactor scale industrial chemical production.

References

1. Crosby, J. R., Laemthong, T., Lewis, A. M., Straub, C. T., Adams, M. W. W. and Kelly, R. M. (2019) Extreme thermophiles as emerging metabolic engineering platforms. *Curr. Opin. Biotechnol.* **59,** 55-64
2. Straub, C. T., Khatibi, P. A., Wang, J. P., Conway, J. M., Williams-Rhaesa, A. M., Peszlen, I. M., Chiang, V. L., Adams, M. W. W. and Kelly, R. M. (2019) Quantitative fermentation of unpretreated transgenic poplar by *Caldicellulosiruptor bescii. Nature Comm.* **10,** 3548)
3. Rubinstein, G. M., Lipscomb, G. L., Williams-Rhaesa, A. M., Kelly, R. M. and Adams, M. W. W. (2020) Engineering the cellulolytic extreme thermophile *Caldicellulosiruptor bescii* to reduce carboxylic acids to alcohols using plant biomass as the energy source. *J. Indust. Micro. Biotech.* **47,** 585-597
4. Straub, C. T., Bing, R. G,, Wang, J. P., Chiang, V. L., Adams, M. W. W. and Kelly, R. M. (2020) Use of the lignocellulose-degrading bacterium *Caldicellulosiruptor bescii* to assess recalcitrance and conversion of wild-type and transgenic poplar. *Biotechnol. Biofuels* **13,** 43
5. Lee, L.L., Crosby, J. R., Rubinstein, G. M., Laemthong, T., Bing, R. G., Straub, C. T., Adams, M. W. W. and Kelly, R.M. (2020) The biology and biotechnology of the genus *Caldicellulosiruptor*: recent developments in Caldi world. *Extremophiles* **24,** 1-15
6. Zhang, K., Zhao, W., Rodionov, D., Rubinstein, G. M., Nguyen, D. N., Tanwee, T. N. N., Crosby, J., Bing, R. G., Kelly, R. M., Adams, M. W. W. and Zhang, Y. (2020) "Genome-scale metabolic model of *Caldicellulosiruptor bescii* reveals optimal metabolic engineering strategies for ethanol production" *mSystems* (submitted 12/2020)
7. Rodionov, D. A., Rodionova, I. A., Rodionov, V. A., Zhang, K., Rubinstein, G. M., Tanwee, T. N. N., Crosby, J., Bing, R. G., Nookaew, I., Basen, M., Brown, S. D., Wilson, C., Klingeman, D. M., Poole, F. L., Zhang, Y., Kelly, R. M. and Adams, M. W. W. (2020) "Transcriptional regulation of biomass degradation and carbohydrate utilization in *C. bescii*" *mSystems* (submitted 12/2020)
8. Straub, C.T., Bing, R.G., Otten, J.K., Keller, L. M., Zeldes, B.M., Adams, M.W.W, and Kelly, R.M (2020) Metabolically engineered *Caldicellulosiruptor bescii* as a platform for producing acetone and hydrogen from lignocellulose. *Biotechnol. Bioengineer*. **117**: 3799– 3808.

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