Energy and Carbon Optimized Conversion of Lignocellulose to Biobased Chemicals by Extreme Thermophiles

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Project Goals: The goal of this project is to establish the two non-model microorganisms *Caldicellulosiruptor bescii* and *Pyrococcus furiosus* as platforms for sustainable production of industrial chemicals using renewable plant biomass. We aim to engineer *C. bescii* and *P. furiosus* to reincorporate CO_2 and H_2 generated during fermentation of lignocellulose as additional sources of carbon and energy, to potentially reach net zero CO_2 emission. Furthermore, *P. furiosus* will be engineered with enzymes from *C. bescii* to allow it to degrade non-pretreated plant biomass. System-wide metabolic and regulatory modeling of both organisms will be used to optimize biomass degradation and desired product yield and selectivity.

The extreme thermophiles *Caldicellulosiruptor bescii* (T_{max} = 90°C) and *Pyrococcus furiosus* (T_{max} = 103°C) share the distinction within the bacterial and archaeal domains, respectively, of being the most thermophilic member having a functional genetic system. Over the last decade, *C. bescii* has been metabolically engineered to produce ethanol, acetone, and various alcohols (1-3) and *P. furiosus* has been engineered to produce lactate, ethanol, and 3-hydrxypropionate (4-6). Fermentation at high temperatures reduces the risk of contamination, facilitates product recovery, minimizes cooling costs, and enhances biomass solubilization (7). These advantages, combined with the native ability of *C. bescii* to deconstruct non-pretreated lignocellulosic biomass (8-11), offer environmentally sustainable, economic platforms to engineer production of industrial products. Our labs have made recent advancements toward strengthening the knowledge-base of *C. bescii* by defining the regulatory pathways of carbohydrate utilization (8) and constructing a genome-scale metabolic model (12). We aim to improve our understanding of *P. furiosus* by applying similar approaches. One aspect of the experimental work is focused on engineering *P. furiosus* with (hemi)cellulases from *C. bescii* to enable its growth on lignocellulosic substrates, since *P. furiosus* can utilize cellobiose but not cellulose or xylan (13). Additional strains of *C. bescii* and *P. furiosus* are under construction to produce industrially-relevant compounds. A key aspect of the engineering strategy is to recycle CO_2 and H_2 generated from lignocellulose fermentation. Target chemicals have been selected strategically to use the CO_2 generated during one biomass-to-bioproduct formation for incorporation into a second bioproduct. This biomass-to-bioproduct conversion will be enhanced by regenerating additional redox cofactors from H_2. To accomplish this experimentally, we will leverage the carbon fixing and hydrogenase enzymes from *P. furiosus* in genetic engineering of *C. bescii*. The genome-scale metabolic models of both *C. bescii* and *P. furiosus* are being used to evaluate each

prospective engineered pathway to enhance production capacity of target products while optimizing carbon and electron fluxes.

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