199. Toward Thermophilic Isobutanol Production in *Clostridium thermocellum*

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Isobutanol is an emerging biofuel that has a comparable energy density to gasoline and is compatible with existing transportation infrastructure. The thermophilic microorganism, *Clostridium thermocellum*, is a potential host for cost-effective biofuel production using cellulose directly as the raw material. We identified all biosynthetic pathway enzymes from pyruvate to isobutanol that are stable at least 50°C. This combination of enzymes produced more than 3 g/L of isobutanol at 50°C when overexpressed in the thermophile, *Geobacillus thermoglucosidasius*. In addition, the *Lactococcus lactis* ketoisovalerate decarboxylase has been functionally expressed in *C. thermocellum* using a plasmid construct; however plasmid stability may be an issue. We also cloned, purified and assayed 14 putative alcohol dehydrogenases from the facultative anaerobe *G. thermoglucosidasius*. Five of which demonstrated the ability to reduce isobutyraldehyde to isobutanol. In particular, Geoth_3237 and Geoth_3823 were found to have high activity using the cofactors NADH and NADPH, respectively. In addition, we have also identified small-scale fermentation conditions to produce isobutanol in wild-type *C. thermocellum*. High concentrations of either cellobiose or cellulose without citrate promote isobutanol production. This result indicates that all of the necessary isobutanol production enzymes are available in the *C. thermocellum* genome, but enzyme identification and improvement will be necessary to improve isobutanol production to desired levels.

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