8. Engineering a New Alcohol Production Pathway Into the Cellulolytic Thermophile Caldicellulosiruptor Bescii

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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 (CBP)]. 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.

A novel metabolic pathway for bioalcohol production was recently engineered into the hyperthermophilic archaeon Pyrococcus furiosus. Insertion of a bacterial alcohol dehydrogenase gene (AdhA) into P. furiosus allows the conversion of glucose to ethanol in a pathway that proceeds through acetate, rather than acetyl-CoA. Acetate is reduced to acetaldehyde, catalyzed by the native aldehyde ferredoxin oxidoreductase (AOR) in P. furiosus. Acetate reduction is driven thermodynamically by the low redox potential of the electron carrier, a 4Fe-ferredoxin. The heterologously expressed AdhA from Thermoanaerobacter strain X514 catalyzes acetaldehyde reduction to ethanol using nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor. Interestingly, due to the broad substrate specificities of both AOR and AdhA, a variety of exogenously added carboxylic acids are also converted to their corresponding alcohols.

The goal of this project is to engineer the new pathway into Caldicellulosiruptor bescii. This thermophilic, anaerobic, gram-positive bacterium utilizes unpretreated plant biomass as a sole carbon source. C. bescii does not contain functional alcohol dehydrogenases but it does possess an AOR homolog. However, this enzyme does not utilize acetaldehyde as a substrate and is of unknown function. AOR is a complex tungstopterin-containing enzyme that requires a plethora of processing proteins for cofactor assembly, all of which are encoded in the C. bescii genome. In a first step, we have expressed the gene encoding P. furiosus AOR in C. bescii and active AOR is produced, demonstrating C. bescii has a functional tungstopterin cofactor biosynthesis pathway. We have now constructed several plasmid vectors containing both AOR and AdhA from a variety of different thermophilic microorganisms that take into account possible differences in their specificities for nicotinamide adenine dinucleotide, NADPH and 4Fe- and 8Fe-ferredoxins. Characterization of the recombinant strains is currently underway. The novel AOR/AdhA pathway may provide benefits over the previously published engineered route to ethanol from acetyl-CoA in bescii because this pathway is potentially redox balanced and energy conserving.