Isolation and Rapid Domestication of Non-Model Microbial Hosts for Biofuels Production

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Many organisms naturally possess complex physiological traits that are of interest for biotechnology research. Often, these traits are challenging to transfer into traditional host organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. Therefore, the ability to identify and rapidly domesticate non-model organisms to harness these traits could usher in a new era of biotechnology where synthetic biology is routinely applied to these organisms. One such complex phenotype is cellulose utilization at low pH. Even in the absence of organic acid fermentation pathways, the CO_2 produced during fermentation requires the use of pH control to maintain neutral conditions. However, so far, only one bacterium, *Acidothermus cellulolyticus*, has been identified that is capable of growing on cellulose at or below pH 5 or lower. Therefore, we are enriching and isolating additional organisms capable of growth on crystalline cellulose at pH 5 and lower. Once identified, these new organisms will be explored for use in consolidated bioprocessing (CBP), the one-pot conversion of cellulose to product without the addition of biomass-degrading enzymes.

No known organisms have all the necessary phenotypes for biomass conversion to fuels and chemicals, so genetic manipulation will be needed to engineer them to have the desired traits. However, non-model organisms are typically unable to be bioengineered due to a lack of available genetic tools and an insufficient foundation of knowledge about the organism. The development of genetic tools is limited largely by the inability to efficiently transform DNA into these organisms. A critical barrier to transformation is DNA restriction-modification systems, which act as a bacterial immune system to cut DNA that is methylated differently than the host. Typically, these systems are comprised of methylation and restriction subunits. To prevent host

death, the cognate DNA methyltransferases recognize the same target sequence as the restriction enzymes, and the methylated DNA is protected from restriction. Therefore, in order to prevent restriction of heterologous DNA, the DNA needs to be methylated in the same manner as the host organism prior to transformation. In order to determine the sites targeted for restriction, we performed methylome analysis on 22 metabolically and phylogenetically diverse organisms of interest in collaboration with the Department of Energy Joint Genome Institute. This information was used to choose methyltransferases for expression in *E. coli* to protect DNA for 16 of these organisms, which has led to improved or first demonstration of genetic transformation in 8 of these organisms thus far. This approach is also being applied to *A. cellulolyticus* and will applied to new isolates that utilize cellulose at low pH. Overall, this work is leading to the development of new bioengineering platform organisms for use in the production of renewable fuels and chemicals, and similar approaches are being taken with other organisms of interest to demonstrate that this is a broadly applicable approach to developing new host organisms for advanced bioengineering.

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