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. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Many organisms naturally possess complex physiological phenotypes 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 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. However, these 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 in 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 in these strains, we performed methylome analysis for organisms of interest in collaboration with the Department of Energy's Joint Genome Institute. This information was used to choose methyltransferases for expression in *E. coli* to protect DNA for *Clostridium clariflavum* and *Megasphaera elsdenii* transformation. For *C. clariflavum*, nine distinct DNA sequences were found to be methylated in the native host. Methyltransferases targeting five of these sites have been expressed in *E. coli* and demonstrated to be functional using methylome analysis on the *E. coli* expression strain. While not methylated completely in the same positions

as the native host, this DNA was used to demonstrate successful transformation of *C. clariflavum*. Similarly, through a combination of methylome analysis and methyltransferase expression in *E. coli*, we demonstrated DNA transformation of two different *M. elsdenii* strains that each have different methylation patterns.

To develop *M. elsdenii* into a new bioengineering platform, we also began addressing the knowledge gap in *Megasphaera*. We sequenced the *M. elsdenii* genome and used this information to build a metabolic reconstruction in the DOE Systems Biology Knowledgebase, which will serve as the foundation for a metabolic model. Transcriptomic data is also helping to elucidate the metabolic pathways used by *M. elsdenii* for the bioconversion of lactic acid and sugars into butyric and hexanoic acids. The genetic tools are now being combined with the new physiological knowledge to engineer *M. elsdenii* to expand the substrate range and to produce new value-added products. 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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