Rational Methyltransferase Expression in *Escherichia coli* for Transformation of New Organisms

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus)* to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Many organisms naturally possess complex physiological traits that are of interest for biotechnology research. The ability to easily harness these traits in their native host could usher in a new era of biotechnology where synthetic biology is routinely applied to these non-standard organisms. However, many of these organisms are unable to be bioengineered due to a lack of available genetic tools. The development of genetic tools is limited in part by the inability to efficiently transform DNA into these organisms. One of the major barriers to successful transformation of bacteria is native DNA restriction-modification systems. DNA restriction-modification systems act as a bacterial immune system to cut DNA that is methylated differently than the host and are typically comprised of methylation and restriction subunits. To prevent host death, restriction enzymes and the cognate DNA methylases recognize the same target sequence. Therefore, in order to overcome restriction, 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, methylome analysis was initially performed for 17 organisms in collaboration with the Department of Energy Joint Genome Institute. This information was used to choose methyltransferases for expression in *E. coli* using a new system for multiple gene integration in *E. coli*. The gene integration system utilizes serine bacteriophage integrases, which enable a single, unidirectional recombination event between two specific DNA sequences, *attB* and *attP,* for stable insertion of DNA into the *E. coli* chromosome. This process of mimicking host methylation patterns successfully allowed for transformation of the type strain of *Clostridium thermocellum*, strain ATCC 27405. The native methyltransferase gene Cthe0519

and a bi-functional Phi3TI methyltransferase were expressed from the *E. coli* chromosome. Plasmid DNA isolated from this methylating *E. coli* strain was then transformed into *C. thermocellum*. A similar approach is being used to demonstrate genetic transformation of other phylogenetically and metabolically diverse organisms. This system will allow for rapid transformation of new organisms to help further bioengineering research.

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