Transplantation of *Cellvibrio japonicus* biomass deconstruction system into *Escherichia coli*

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Project Goals: The Great Lakes Bioenergy Research Center (GLBRC) aims to identify and overcome key barriers to the sustainable conversion of lignocellulosic biomass to biofuels. An organism capable of both digesting plant biomass and synthesizing biofuels could be a useful tool for manufacturing next-generation biofuels. One goal is to construct microbial strains that can both release of sugars from cellulose and hemicellulose and convert them to ethanol or other biofuels. To this end, we are working to imbue *Escherichia coli* with the lignocellulolytic capabilities of the plant-degrading γ-proteobacterium *Cellvibrio japonicus*. We are identifying the core set of glycosyl hydrolases necessary for lignocellulose breakdown by *C. japonicus*, and expressing these enzymes in *E. coli* along with the genes encoding the Type II secretion system necessary for their secretion.

A key barrier to economically sustainable lignocellulosic biofuels is the difficulty in releasing sugars from lignocellulose. One approach to overcoming this barrier is to engineer the expression of genes encoding glycosyl hydrolases capable of biomass deconstruction into a biofuel-producing microbe. *E. coli* offers an excellent platform in which to engineer various metabolic pathways to biofuels, but lacks a native ability to produce and secrete relevant glycosyl hydrolases. Our studies have shown that the evolutionarily related γ-proteobacterium *Cellvibrio japonicus* can deconstruct the plant cell walls of bioenergy-relevant substrates such as corn stover and switchgrass. Examination of the genome of *C. japonicus* has identified 154 candidate glycosyl hydrolase genes potentially involved in lignocellulose degradation. However, the role of most of these genes in plant cell wall degradation has not been defined.

To identify the core set of lignocellulases necessary for cell wall deconstruction, we used global gene expression profiling to investigate how *C. japonicus* degrades lignocellulose. Although *C. japonicus* encodes a large number of candidate glycosyl hydrolases, fewer than half were found to be expressed during growth in the presence of corn stover. Transplantation of a subset of these genes into *E. coli* allowed *E. coli* to degrade cellulose and xylan, and to grow on cellulosic carbon sources such as carboxymethyl cellulose and Avicel. Collectively, these results demonstrate the potential of this approach to identify lignocellulase genes for engineering of CBP organisms, and to contribute to our overall knowledge of microbial cell wall degradation.

C. japonicus secretes much of its glycosyl hydrolase activity using a Type II secretion system (1). We transplanted the *gsp* operon encoding the *C. japonicus* Type II secretion system into *E. coli* to determine whether it could allow delivery of *C. japonicus* enzymes expressed in *E. coli* to extracellular substrates. We found that the *gsp* genes were expressed by *E. coli*, and that co-expression with certain enzymes lead to general leakage of periplasmic proteins. However, the *C. japonicus gsp* operon alone, while expressed, did not generate a functional Type II secretion system. This provides an ideal system with which we can now screen for components of *C. japonicus* necessary for functional transplantation of its Type II secretion system.

References

1. Gardner JG, Keating DH (2010). Appl Environ Microbiol 76 (15):5079-5087.

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