Functional Characterization and Regulatory Modeling of Lignocellulose Deconstruction in the Saprophytic Bacterium *Cellvibrio japonicus*

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Project Goals: Understanding polysaccharide degradation by microbes is of great importance to unraveling environmental processes driving global nutrient cycles, determining gut microbiota nutritional contributions, and alleviating bottlenecks in renewable fuel and chemical production. However, one current knowledge gap is how these microbes are able to degrade and consume what is effectively an unreactive and inert substrate (lignocellulose). To that end, completion of this project will facilitate the establishment a fundamental systems-level model of lignocellulose deconstruction by saprophytic soil bacteria. Additionally, over the course of this project we will identify and characterize novel enzymes that have the potential to accelerate the advancement of renewable fuel and chemical technologies.

Substantial engineering of industrially relevant bacteria allows a synthetic metabolism in these microbes to make desirable compounds. However, one challenge that still exists is obtaining low cost substrates that feed into this synthetic metabolism. Cellulosic materials represent a deep reservoir of sugars, however they are locked in a recalcitrant polymeric form. Decades of enzymatic studies have sought to overcome the recalcitrance of environmental polysaccharides such as lignocellulose. Recently several biochemical studies have commented that *in vivo* studies of recalcitrant polysaccharide degradation will be required to fully understand the process, as exclusively *in vitro* studies will not necessarily identify more efficient enzymes, especially in a physiologically or biotechnologically relevant context [1-3]. Therefore, returning to fundamental studies of environmental bacteria that are proficient at lignocellulose degradation presents a promising area of study. Novel enzymes with desirable properties uncovered by this approach can then be further enhanced with synthetic biology techniques for renewable fuel and chemical production.

We have employed systems approaches and interdisciplinary studies to characterize in a physiologically relevant manner the cellulose degradation capabilities in saprophytic bacteria [4]. Our approach is comprehensive and incorporates a diversity of techniques. Briefly, we use transcriptomics (RNAseq) to determine both up-regulated and highly constitutive genes under conditions of interest (*i.e.* degrading lignocellulose). This approach yields a gene set to study with classical bacterial genetics. Using an in-frame deletion strategy developed by our group [5], we can evaluate sets of genes in a physiologically relevant manner and determine genes with essential function. This approach has also uncovered novel genes of previously unknown function [4]. Enzyme kinetic analysis further characterizes essential genes, while determining if they are candidates for industrial processes. Completing our analysis toolkit are computational methods for determining suites of co-regulated genes and regulatory networks. A synthesis of these data results in a comprehensive and predictive model of polysaccharide degradation by saprophytic bacteria.

Here we present our recent progress examining recalcitrant polysaccharide degradation using the model saprophytic bacterium *Cellvibrio japonicus*. We will discuss our methods to use exclusively physiologically relevant substrates (*e.g.* minimally processed corn stover or switchgrass) to discover novel enzyme targets. Additionally, we will describe our approach to modeling the complex regulatory networks required to detect and consume environmental polysaccharides.

References

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