Saprophytic Bacterium *Cellvibrio japonicus* Has Selective CAZyme Requirements During Physiologically Relevant Hemicellulose Degradation

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Project Goals: Completion of the project will identify and characterize the physiologically relevant carbohydrate active enzymes required to consume the polysaccharides found in lignocellulose by the saprophytic soil bacterium *Cellvibrio japonicus*. Additionally, over the course of the project the utility of these enzymes, including assessment of novel functions, will be evaluated for biotechnology applications.

Despite substantial published data on the structural and enzymatic parameters of recalcitrant polysaccharide deconstruction, there is much less work on understanding how carbohydrate active enzymes (CAZymes) exert their effects *in vivo*. We have used the soil bacterium *Cellvibrio japonicus* to generate a systems-level understanding of lignocellulose utilization, and here we report our progress on determining the essential factors for hemicellulose degradation. Specifically, via RNAseq seven CAZyme genes were significantly up-regulated during growth on xylan. A comprehensive gene deletion strategy determined that only one of these genes was essential for xylan utilization. Expression of this gene in *E. coli* generated an engineered strain that was proficient utilizing xylo-oligosaccharides. Other significant findings were that *C. japonicus* uses both secreted and membrane-bound CAZymes for hemicellulose degradation degradation. This strategy deviates from how the bacterium degrades cellulose. Finally, we found that a single critical CAZyme is necessary for soluble hemicellulose oligosaccharide utilization.

Publications

1. Blake AD, Beri NR, Guttman HS, Cheng R, and Gardner JG. 2018. The complex physiology of *Cellvibrio japonicus* xylan degradation relies on a single cytoplasmic β-xylosidase for xylo-oligosaccharide utilization. *Molecular Microbiology*. 107:610-22.

2. Nelson CE, Attia MA, Rogowski A, Morland C, Brumer H, and Gardner JG. 2017. Comprehensive functional characterization of the Glycoside Hydrolase Family 3 enzymes from *Cellvibrio japonicus* reveals unique metabolic roles in biomass saccharification. *Environmental Microbiology*. 19:5025-39.

3. Nelson CE, Rogowski A, Morland C, Wilhide JA, Gilbert HJ, and Gardner JG. 2017. Systems analysis in *Cellvibrio japonicus* resolves predicted redundancy of β-glucosidases and determines essential physiological functions. *Molecular Microbiology*. 104:294-05.

4. Nelson CE, Beri NR, and Gardner JG. 2016. Custom fabrication of biomass containment devices using 3-D printing enables bacterial growth analyses with complex insoluble substrates. *Journal of Microbiological Methods*. 130:136-43.

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