

## **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. 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  $\beta$ -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  $\beta$ -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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