

## Using Systems Biology to Untangle the Complex Physiology of Bacterial Xylan Utilization

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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.**

Lignocellulose degradation by microbes plays a central role in global carbon cycling, human gut metabolism, and renewable energy technologies. While considerable effort has been put into understanding the biochemical aspects of lignocellulose degradation, much less work has been done to understand how these enzymes work in an *in vivo* context. Here, we report a systems level study of xylan degradation in the saprophytic bacterium *Cellvibrio japonicus*. Transcriptome analysis indicated seven genes that encode carbohydrate active enzymes were up-regulated during growth with xylan containing media. In-frame deletion analysis of these genes found that only *gly43F* is critical for utilization of xylo-oligosaccharides, xylan, and arabinoxylan. Heterologous expression of *gly43F* was sufficient for the utilization of xylo-oligosaccharides in *Escherichia coli*. Additional analysis found that the *xyn11A*, *xyn11B*, *abf43L*, *abf43K*, and *abf51A* gene products were critical for utilization of arabinoxylan. Furthermore, a predicted transporter (CJA\_1315) was required for effective utilization of xylan substrates, and we propose this unannotated gene be called *xntA* (xylan transporter A). Our major findings are (i) *C. japonicus* employs both secreted and surface associated enzymes for xylan degradation, which differs from the strategy used for cellulose degradation, and (ii) a single cytoplasmic  $\beta$ -xylosidase is essential for the utilization of xylo-oligosaccharides.

### Publications

1. Garcia CA, Narrett JA, Gardner JG. 2019. Complete Genome Sequences of *Cellvibrio japonicus* Strains with Improved Growth When Using  $\alpha$ -Diglucosides. *Microbiology Resource Announcements*. 8(44).
2. 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.
3. 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.
4. 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.

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