Comprehensive Functional Characterization of the Glycoside Hydrolase Family 3 Enzymes from *Cellvibrio japonicus* Reveals Unique Metabolic Roles in Biomass Saccharification

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Project Goals: This project will generate a predictive systems-level model of lignocellulose deconstruction for the saprophytic soil bacterium *Cellvibrio japonicus*. In addition, this project will characterize novel Carbohydrate Active Enzymes and probe their utility for biotechnology applications, such as renewable fuels and commodity chemicals.

Lignocellulose degradation is central to the carbon cycle and renewable biotechnologies. The xyloglucan (XyG), β(1→3)/β(1→4) mixed-linkage glucan (MLG), and β(1→3) glucan components of lignocellulose represent significant carbohydrate energy sources for saprophytic microorganisms. The bacterium *Cellvibrio japonicus* has a robust capacity for plant polysaccharide degradation, due to a genome encoding a large contingent of Carbohydrate-Active Enzymes (CAZymes), many of whose specific functions remain unknown. Using a comprehensive genetic and biochemical approach we have delineated the physiological roles of the four *C. japonicus* Glycoside Hydrolase Family 3 (GH3) members on diverse β-glucans. Despite high protein sequence similarity and partially overlapping activity profiles on disaccharides, these β-glucosidases are not functionally equivalent. Bgl3A has a major role in MLG and sophorose utilization, and supports β(1→3) glucan utilization, while Bgl3B underpins cellulose utilization and supports MLG utilization. Bgl3C drives β(1→3) glucan utilization. Finally, Bgl3D is the crucial β-glucosidase for XyG utilization. This study not only sheds the light on the metabolic machinery of *C. japonicus*, but also expands the repertoire of characterized CAZymes for future deployment in biotechnological applications. In particular, the precise functional analysis provided here serves as a reference for informed bioinformatics on the genomes of other *Cellvibrio* and related species.

Publications

1. Gardner JG**.** 2016. Polysaccharide degradation systems of the saprophytic bacterium *Cellvibrio japonicus*. *World Journal of Microbiology and Biotechnology*. 32:121-32.

2. 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.

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.

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