

Systems analysis in *Cellvibrio japonicus* resolves predicted redundancy of β -glucosidases and determines essential physiological functions

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Project Goals: This project aims to facilitate the establishment a fundamental systems-level model of lignocellulose deconstruction by saprophytic soil bacteria. 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.

Degradation of polysaccharides forms an essential arc in the carbon cycle, provides a percentage of our daily caloric intake, and is a major driver in the renewable chemical industry. Microorganisms proficient at degrading insoluble polysaccharides possess large numbers of carbohydrate active enzymes, many of which have been categorized as functionally redundant. Here we present data that suggests that carbohydrate active enzymes that have overlapping enzymatic activities can have unique, non-overlapping biological functions in the cell. Our comprehensive study to understand cellodextrin utilization in the soil saprophyte *Cellvibrio japonicus* found that only one of four predicted β -glucosidases is required in a physiological context. Gene deletion analysis indicated that only the *cel3B* gene product is essential for efficient cellodextrin utilization in *C. japonicus* and is constitutively expressed at high levels. Interestingly, expression of individual β -glucosidases in *Escherichia coli* K-12 enabled this non-cellulolytic bacterium to be fully capable of using cellobiose as a sole carbon source. Furthermore, enzyme kinetic studies indicated that the Cel3A enzyme is significantly more active than the Cel3B enzyme on the oligosaccharides but not disaccharides. Our approach for parsing related carbohydrate active enzymes to determine actual physiological roles in the cell can be applied to other polysaccharide-degradation systems.

Publications

1. Nelson C.E. and Gardner J.G. 2015. In-frame deletions allow functional characterization of complex cellulose degradation phenotypes in *Cellvibrio japonicus*. *Applied and Environmental Microbiology*. 81(17): 5968-75.
2. Gardner JG. 2016. Polysaccharide degradation systems of the saprophytic bacterium *Cellvibrio japonicus*. *World Journal of Microbiology and Biotechnology*. 32(7):121-32.
3. 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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