

Microbial Valorization of Lignin: Using Bacteria and Their Enzymes to Develop Biological Systems for Depolymerizing Lignin

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Project Goals: As part of the Great Lakes Bioenergy Research Center, we are working on developing biological systems for converting lignin from plant biomass into valuable chemicals. Lignin is a hetero-polymer of aromatic units that makes up ~25% of lignocellulosic plant biomass. While the individual aromatic compounds that make up lignin could have potential value for several industries, lignin is difficult to break down. One aspect of our work is studying bacteria and their enzymes for development into systems to help depolymerize lignin. In this work, we have identified the soil bacterium *Novosphingobium aromaticivorans* as being one of the best organisms thus far characterized at breaking the β -aryl ether bond, which is the most common linkage between aromatic units in natural lignin. We have used *N. aromaticivorans* to identify and characterize enzymes involved in breaking the β -aryl ether bond, and have used enzymes from *N. aromaticivorans* and other bacteria to develop an *in vitro* system for depolymerizing bona fide plant-derived lignin.

There is economic and environmental interest in using renewable resources as raw materials for production of chemicals that are currently derived from fossil fuels. Lignin, a hetero-polymer composed of several aromatic subunits, can make up ~25% (dry weight) of vascular plant cell walls (1), making it one of the most abundant renewable organic materials on Earth. In its polymeric form, lignin has limited economic uses- it is generally discarded or burned to generate energy- but the aromatic compounds that make up lignin could potentially be used in the biofuel, chemical, cosmetic, food, and pharmaceutical industries (2). However, due largely to its irregular, covalently bonded structure, lignin has historically been difficult to depolymerize. Consequently, intensive efforts are currently aimed at developing chemical, enzymatic, and hybrid methods for deriving simpler and lower molecular weight products from lignin (2).

While the aromatic units in lignin are linked together via several classes of covalent bonds, the β -aryl ether (β -O-4) bond typically constitutes >50% of all the linkages (3), making it an important target in developing systems for depolymerizing lignin. The β -etherase pathway, found in some sphingomonad bacteria, is a promising biological route for cleaving the β -aryl ether bond. In this metabolic pathway, the ether bond between aromatic units is broken by replacement with a thioether bond involving glutathione (2). Several species are known that can

perform this pathway, and many of the enzymes required for the reactions of the pathway have been identified and characterized.

We tested several additional bacteria and found that *Novosphingobium aromaticivorans* can cleave the β -aryl ether bond of a dimeric aromatic compound at one of the fastest rates thus far reported, making it a good candidate for using as a cellular chassis for lignin depolymerization systems. We have also used *N. aromaticivorans* to study various aspects of the β -etherase pathway, including identifying the enzymes involved in the pathway. Through this work, we identified a previously uncharacterized enzyme that is integral to the pathway: a glutathione-S-transferase that removes glutathione from both stereoisomers of one of the intermediates in the pathway. This work has important implications for not only lignin depolymerization, but also for understanding glutathione-S-transferases and the reactions that they can catalyze more broadly.

While *N. aromaticivorans* could be used to cleave the β -aryl ether bond of plant-derived lignin oligomers, there may be limits to the size of the oligomers that can be transported into the cells for cleavage. We have therefore also developed an *in vitro* system using the minimal set of purified enzymes necessary to break the racemic β -aryl ether bond. Along with β -etherase pathway enzymes from *N. aromaticivorans* and a similar bacterium (*Sphingobium* sp. SYK-6), our system includes a glutathione reductase from *Allochromatium vinosum*, which recycles the cofactors NAD⁺ and glutathione required for the pathway, thus limiting the expense of the system by eliminating the need to continuously supply fresh cofactors. We tested this system on bona fide plant-derived lignin and successfully cleaved monomeric aromatic compounds from the lignin polymer. This work is important for the use and future optimization of these bacterial enzymes for industrial level biotechnological applications designed to derive high-value monomeric aromatic compounds from lignin.

References

1. U.S. DOE (2015) Lignocellulose Biomass for Advanced Biofuels and Bioproducts: Workshop Report, DOE/SC-0170. U.S. Department of Energy Office of Science. Available at: <http://genomicscience.energy.gov/biofuels/lignocellulose/>
2. Gall DL, Ralph J, Donohue TJ, Noguera DR (2017) Biochemical transformation of lignin for deriving valued commodities from lignocellulose. *Curr Opin Biotechnol* 45, 120-126.
3. Adler E: Lignin chemistry - past, present and future. *Wood Science and Technology* 1977, **11**:169-218.

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