Bacterial lignin degradation

Lignin is a heterogeneous aromatic polymer that comprises ~25% of the land-based biomass. It occurs in tight association with cellulose and hemicellulose to form lignocellulose, the rigid, recalcitrant material in woody plants. Lignocellulose is touted as a sustainable source of energy and biomaterials. Lignin alone is of burgeoning interest as a feedstock for aromatic chemicals, resins and carbon fibres. Nevertheless, lignocellulose-derived products are not economically viable due in part to the energy-intensive processes used to deconstruct biomass. Biocatalysts offer a greener, more energy-efficient means to extract increased value from biomass. Potential applications are illustrated by our mutant of *Rhodococcus jostii* RHA1 (RHA1) which accumulates vanillin when incubated with lignocellulose. Understanding lignin catabolic enzymes and pathways will provide insight into processes underlying carbon cycling in forest ecosystems and will facilitate the development of biocatalysts to transform lignocellulose.

Lignin degradation occurs in two stages: (a) non-specific, extracellular depolymerization to aryl and biaryl compounds such as b-aryl ethers; and (b) the mineralization of these latter by specific
Catabolic enzymes and pathways. The best characterized depolymerization enzymes are those secreted by white rot fungi: lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. LiP and MnP oxidize small compounds or metals that function as mediators for lignin oxidation. For example, MnP oxidizes Mn2+ to Mn3+, which in turn either oxidizes lignin’s phenolic structures or generates peroxy radicals to oxidize its non-phenolic structures. However, fungal enzymes have limited industrial utility as they are difficult to produce in commercially viable amounts. By contrast, bacterial enzymes are relatively easy to produce.

Lignin-degrading bacteria have long been overlooked. However, at least three different classes have now been identified and several bacterial enzymes that depolymerize lignin have been recognized. The latter include ALiP-P3, a peroxidase from a streptomycete, reported to cleave -aryl ethers, and the putative laccases and peroxidases of Enterobacter lignolyticus SCF1. Nevertheless, the first characterized bacterial ?ligninase? was DypB from RHA1. DypB belongs to a family of heme proteins called dye-decolorizing peroxidases (DyP), and oxidizes lignin and Mn2+. In the past year, DyP2 from Amycolatopsis sp. 75iv2 was also shown to oxidize Mn2+ and lignin model compounds. More recently, we have generated an active site variant of DypB, N246A, that oxidizes Mn2+ more efficiently than wild-type DypB, and transforms Kraft lignin to syringaldehyde and other monoaryls. Nevertheless, the few bacterial ligninases characterized to date are less efficient than the fungal ones.

The aromatic compounds resulting from lignin depolymerization are catabolized primarily by bacteria: fungi depolymerize lignin to access cellulose as a growth substrate. Bacterial aromatic catabolic pathways have been extensively studied, as exemplified by those of Pseudomonas putida KT2440 and RHA1. Briefly, aromatic compounds are degraded by peripheral aromatic pathways to a limited number of intermediates that feed into central aromatic pathways. The catabolism of lignin-derived vanillin by RHA1 illustrates this principle. Thus, vanillin dehydrogenase and vanillate O-demethylase comprise a peripheral pathway that transforms vanillin to protocatechuate. This intermediate, which is also generated in phthalate and 4-hydroxybenzoate catabolism, is degraded to central metabolites by the ?-ketoadipate pathway, a central aromatic pathway. The catabolism of lignin-derived aromatic compounds has also been studied in Sphingobium sp. SYK-6. For example, 2,2?-dihydroxy-3,3?-dimethoxy-5,5?-dicarboxybiphenyl (DDVA) is O-demethylated by LigX to produce 2,2?,3-trihydroxy-3?-methoxy-5,5?-dicarboxybiphenyl (OH-DDVA). The latter is ring-cleaved by a dioxygenase, LigZ, to produce a meta-cleavage product (MCP), 4,11-dicarboxy-8-hydroxy-9-methoxy (DCHM-) 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA). DCHM-HOPDA is hydrolyzed by LigY, yielding 5-carboxyvanillate (5CVA), which is decarboxylated to vanillate by LigW. Nevertheless, the catabolism of lignin-derived compounds has not been fully elucidated and the catalytic mechanism of key enzymes is unknown.

Significance. The research on bacterial lignin degradation enzymes will yield fundamental insights into important classes of enzymes. The targeted enzymes are important to the global carbon cycle, particularly in forest ecosystems. Related enzymes are implicated in Alzheimer’s, immune function, vision and other processes. On a more general level, the research will provide insights into the function of metals in biological systems, O2-activation and protein structure:function relationships. On a practical level, the research facilitates the engineering of enzymes and bacteria for green chemistry applications. Woody biomass has considerable potential as a sustainable alternative to petroleum as a feedstock for high-value products.
Effective lignin-transforming biocatalysts would help develop this potential and contribute to revitalizing a struggling forestry industry.

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