Cross-kingdom Comparative Genomics of Aromatic Catabolic Pathways in Fungi and Bacteria

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The overall goal of this project is to test the hypothesis that white-rot fungi can simultaneously depolymerize lignin extracellularly and catabolize depolymerization products intracellularly as carbon and energy sources. The results from this project will lead to improved understanding of lignin utilization by white-rot fungi, and enable identification of promising fungal strains for lignin catabolism and valorization. As part of this effort, we have conducted a comparative genomic study to investigate a diversity of aromatic catabolic pathways in the fungal and bacterial kingdoms. Furthermore, we have combined genomic and phylogenetic approaches to decipher the evolution of certain aromatic catabolic enzymes and discover new classes of enzymes, which represents a foundation for future biochemical and molecular genetic studies.

Although lignin is the second most abundant polymer in plant biomass, its upgrading remains a major hurdle in biorefining due to its recalcitrance and structural heterogeneity. To overcome this challenge, the concept of biological funneling has emerged as an approach to convert heterogeneous mixtures of lignin-derived monomeric aromatic compounds – from various lignin deconstruction processes – to single chemicals by using engineered microbes. For this purpose, knowledge in aromatic catabolic pathways is necessary to design microbes with (1) enhanced substrate diversity utilization, (2) conversion efficiency, and (3) tailored metabolic pathways to produce the desired products (Johnson et al., 2019). Aromatic catabolic pathways have been thoroughly described in several bacteria such as *Pseudomonas putida*, *Burkholderia* sp. SJ98, *Sphingobium* sp. SYK-6, and *Rhodococcus jostii*. However, knowledge of the corresponding aromatic catabolic pathways in white-rot fungi (WRF) is quite limited, even though WRF are known to be the most efficient lignin-degrading organisms in nature (del Cerro et al., 2021).

To reveal the distribution and diversity of aromatic catabolic pathways in WRF, we performed a large-scale comparative genomic and phylogenetic study across the bacterial and fungal kingdoms. First, we selected functionally characterized enzymes from bacteria and recently validated enzymes from WRF (Kijpornyongpan et al., manuscript in preparation) involved in (1) pre-cleavage modifications of aromatic compounds (i.e. oxidative and non-oxidative decarboxylases and aromatic hydroxylases), (2) ring-cleavage (i.e. intradiol and extradiol dioxygenases), and (3) post-cleavage modifications (i.e. oxidoreductases, 3-oxoacid CoA transferases, and thiolases). Second, we examined the protein domains in each enzyme selected for this study through InterProScan, and we selected protein domains with descriptions directly related to the catabolism of aromatic compounds as proxy domains to define a potential capability to modify and/or catabolize aromatic compounds. Third, we sampled 255 bacterial genomes and 317 fungal genomes, representing different lineages and nutritional modes across

the trees of life in these two kingdoms. For each genome, we performed genome-wide protein domain searches and gene homology assessment to identify proteins with proxy domains related to the catabolism of aromatic compounds. Apart from pathway and enzyme discovery, these analyses were also designed to determine if there are distribution patterns or associations between the abundance of aromatic catabolic enzymes and bacterial and fungal lineages and/or their nutritional modes. Finally, we performed phylogenetic analyses to understand the evolution of specific aromatic catabolic enzymes.

Based on the distribution of enzyme domains, we found that fungi have a higher conservation of genes that encode for aromatic catabolic enzymes – in particular intradiol dioxygenases, phenol hydroxylases, and 3-oxoacid CoA transferases – compared to bacteria. Our association analyses indicated that microbes that are either known to utilize aromatic compounds as a carbon source or classified as plant-associated also have a higher abundance of aromatic catabolic enzymes compared to other microbes found in environment or known as animal-associated. In addition, our findings highlighted the evolution of intradiol dioxygenases: specifically, we found separate origins of catechol dioxygenases and 3,4-protocatechuate dioxygenases in bacteria and fungi. The gene tree phylogeny suggested that hydroxyquinol dioxygenases are the most common type of intradiol dioxygenases found in fungi. We also identified a novel class of intradiol dioxygenases, which likely function extracellularly based on the presence of signal peptides. Taken together, this work provides new insights related to the catabolism of aromatic compounds in different groups of bacteria and fungi which could enable the discovery of novel microbes, pathways, and enzymes to improve the 'funnel' to convert lignin-derived aromatic compounds to value-added products.

References

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