Title: A gene-editing system for large-scale fungal phenotyping in a model wood decomposer

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Project Goals: Our goal is to combine systems biology approaches and gene-editing to develop a high-throughput genetic platform for large-scale phenotyping in a model wood decomposer fungus relevant to the DOE mission area. We will develop an efficient, CRISPR/Cas9-based gene-editing platform with model brown rot fungi, discovering and validating at a large scale the genetic functions of key wood-decaying gene pathways identified by network analysis. By this project, we expect to provide stand-alone tools and resources for discovering novel fungal genetic features that can also be used in combination to advance relevant plant biomass conversion research in the post-genomic era.

Abstract:

Wood decay fungi offer industrially-relevant pathways to extract carbohydrates from lignocellulose, and their mechanisms have broad relevance to global carbon cycling. Among these organisms, white rot-type fungi use ligninolytic enzymes (e.g., lignin peroxidases) to break down the lignin barrier, while brown rot fungi use non-enzymatic reactive oxygen species (ROS) mechanisms to modify lignin and selectively extract sugars.[1, 2] Brown rot fungi evolved from white rot ancestral lines multiple times and, from a process efficiency standpoint, they represent a pathway 'upgrade' to approaches that mimic white rot. Brown rot is generally faster than white rot, the mechanism selectively releases soluble sugars by leaving lignin relatively intact as a by-product (value addition).[1, 2] These fungi achieved this, evolutionarily, by contracting rather than enhancing their repertoire of carbohydrate-active enzymes (CAZys), shedding ~65% of their ancestral CAZy genes, on average.[3, 4, 5] Although DOE mission-relevance is clear, and we have made major genomically-informed advances, progress is limited by an inability to manipulate genes in any brown rot fungal strain.

Using multi-omics tools, our team has been leading the research of dissecting this brown rot mechanism 'upgrade'. 1) We reported the first brown rot genome in *Postia placenta* (now *Rhodonia placenta*) in PNAS in 2009.[3] 2) We then used phylogenomics to discover the genetic inventories unique to brown rot fungi in Science in 2012[4] and in PNAS in 2014[5]. 3) More recently, we used functional genomics to further elucidate a staggered 'two-step' (i.e., oxidation-then-hydrolysis) gene regulation model for brown rot in PNAS in 2016 and in mBio in 2019.[6, 7] Although these genomic studies have greatly advanced our understanding of brown rot, its

genetic basis remains uncharacterized and unharnessed. This is, to a large extent, due to the lack of a robust genome editing tool – these fungi have one of the most mission-relevant mechanisms for deconstruction, and we cannot currently engineer their genomes to learn *how* they work.

Targeted gaps are remaining for understanding the brown rot genetic mechanism. *First*, multi-omics approaches have advanced our knowledge of the 'two-step' mechanism,[6, 7] but the functions of genes involved remain unverified and ambiguous. *Second*, brown rot fungi have adapted distinct gene regulatory mechanisms to precisely control and consolidate the two steps during wood decay,[8, 9] but little is known about this process. This limits our ability to refer to brown rot gene functions from other fungi with known decay pathways. *Third*, the majority of genes identified by multi-omics are of hypothetical/unknown function, [5, 6, 7] leaving major gaps for discovering novel functional genes.

This project will integrate systems biology, genome-editing, and network modeling to address these key gaps. Our **objective 1** is to optimize a CRISPR/Cas9-mediated gene-editing system based on the established genetic manipulation platform in brown rot fungi, and use it for targeting genes that have been isolated by multi-omics studies. This will generate a single-gene mutant library for phenotypic studies. Our **objective 2** is to build an extensive carbon utilization network using transcriptomic analyses and network modelling, discovering the distinctive gene regulation features adapted by brown rot. Leveraging this objective, we will also complement the gene pool for large-scale phenotypic screening. Finally, the **objective 3** aims to develop a pipeline to use the multiplexing sgRNA library for genome-editing, building the mutant library for large-scale phenotypic screens. This will allow us to rapidly link genotype to the phenotypes that enable brown rot efficacy.

With this project, we anticipate to provide stand-alone tools and resources to elucidate fundamental microbial processes relevant to DOE mission area, advancing new engineering designs for lignocellulose bioconversion.

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