Heterologous Production of Lignin Modifying Enzymes in *Aspergillus niger*

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Project Goals: The fungal biotechnology group at JBEI is focused on developing fungal expression systems for industrial scale production of enzymes related to biofuel production. Specifically, we are expanding the capacity of heterologous expression hosts to produce complex metalloproteins involved in lignin depolymerization.

A desire to move away from fossil fuels toward more sustainable, renewable, or carbon-neutral sources has motivated the search for alternative feedstocks. Lignocellulosic biomass is an attractive option due to its abundance, low cost, and high sugar content. However, the high cost and limited availability of the enzyme cocktails required for efficient biomass conversion are significant barriers to the adoption of lignocellulosic biomass as a feedstock to produce biofuels and other value-added products. Filamentous fungi have an astounding capacity to secrete digestive enzymes into their extracellular environment through the presence of efficient systems for the transcription, translation, folding, and secretion of polypeptides. These features have led filamentous fungi to be exploited for the manufacturing of homologous and heterologous proteins for the chemical, pharmaceutical, and biofuel industries. Efficient heterologous protein production often requires multilevel optimization strategies for both the protein and the expression host as factors limiting production are often protein specific and bottlenecks can exist at any stage from transcription and translation to folding and secretion. We are developing fungal host systems for the expression and characterization of lignin modifying enzymes (LME) in the filamentous fungus *Aspergillus niger*. More specifically, we are focusing on the post-translational bottlenecks that are known to limit, in terms of quantity and quality, heterologous protein production. We will present the engineering strategies used to build a fungal expression system for LME, and the plan for genetically engineering key components of the secretory pathway with the aim of enhancing heterologous protein production. The development of efficient expression systems and hosts to identify, characterize, and enhance the expression levels, yields, and activities of heterologous LME is critical to the successful adoption of lignocellulosic biomass into the new bio-economy.

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