125. Development of *Aspergillus niger* as a host for hyperproduction of thermophilic cellulases

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Enzymatic saccharification of plant biomass involves the depolymerization of recalcitrant cell wall structures composed primarily of cellulose, hemicellulose and lignin. In order to efficiently liberate fermentable sugars from the plant cell wall, the biomass must be pretreated to reduce recalcitrance using methods such as dilute acid, ammonia fiber expansion (AFEX), or the use of certain ionic liquids (ILs). Specific ILs have proven to be excellent pretreatment reagents, but unfortunately the common classes of IL's, such as 1-ethyl-3-methylimidazolium acetate [C_2mim][OAc], can inhibit downstream saccharification by commercially available cellulase enzyme mixtures. To overcome this problem, researchers at JBEI have leveraged an apparent correlation between IL-tolerance and thermostability of certain cellulase enzymes to develop an IL-tolerant cellulase cocktail, called JTherm. The JTherm cocktail is composed of two recombinant enzymes, a β-glucosidase (BG) and a cellobiohydrolase (CBH) derived from thermophilic bacteria, and a mixture of native thermophilic endoglucanases produced by a bacterial consortia. In order to produce these IL-tolerant enzymes in a more efficient manner, we are attempting to develop *Aspergillus niger* into a high-titer expression system. *A. niger* was selected as the target host because of its proven ability to secrete high-titers of extracellular protein, but the biological mechanisms at a fundamental level remain unknown. We have therefore sought to generate a fundamental understanding of heterologous fungal secretion pathways so that we can translate this information into strategies to engineer *A. niger* to produce high-titers of heterologous enzymes for further enzyme optimization and mixture development. To initiate this work, we transformed *A. niger* with several IL-tolerant enzymes, many of which expressed well and had similar properties compared to enzymes expressed in *E. coli*. We then used these strains to initiate a variety of "–omics" approaches that will enable us to deepen our understanding of enzyme secretion and develop enzyme hyperproduction strains: 1) utilize a forward genetics approach to generate hyperproduction mutants of heterologous IL-tolerant enzymes followed by high-throughput sequencing to identify the mutations; 2) sequence the genomes of existing industrial enzyme hyper-production strains to identify the genomic alterations responsible for their hyper-production phenotypes; 3) develop a reverse genetics platform to stack traits from hyper-production strains/mutants into a single production host; 4) utilize transcriptomic and proteomic analysis to determine the systemic response of *A. niger* to heterologous enzyme production to aid in strain engineering and to identify genetic "parts" that can be used to construct efficient expression constructs. The primary goal of this genome-scale analysis is to gain sufficient information to enable the development of bioengineering strategies that efficiently and selectively increase the expression of heterologous enzymes, in particular enzymes that are used to develop technologies for the conversion of lignocellulosic biomass to fungible advanced biofuels.