

134. Heterologous Expression of Ionic Liquid Tolerant Cellulases in *Aspergillus niger*

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Project Goals: Our objective is to understand the mechanisms that drive high titer enzyme production in *A.niger* and use this knowledge to further its development as a heterologous expression host for high titer production of designer cellulase cocktails.

Cheap and efficient deconstruction of plant biomass is critical for the successful commercialization of lignocellulosic biofuels. Biomass pretreatment with ionic liquids (ILs) has been demonstrated to dramatically increase saccharification efficiency under reduce cellulase enzyme loadings, and therefore shows great promise in this regard. However, some ILs that are good for pretreatment, such as 1-ethyl-3-methylimidazolium acetate, strongly inhibit commercial cellulase enzymes, and therefore excessive amounts of water are required to remove the IL from the biomass prior to saccharification. To address this issue, several IL-tolerant bacterial cellulases have been discovered and assembled into an IL-tolerant cellulase mixture, called JTherm, capable of hydrolyzing pretreated biomass in the presence of 1-ethyl-3-methylimidazolium acetate.

Currently, we are attempting to produce high titers of these IL-tolerant enzymes in *Aspergillus niger* to enable further research and to demonstrate commercial viability of this technology. *A. niger* is an ascomycete filamentous fungus commonly used in industry to produce citric acid a high titers of the glycoside hydrolase glucoamylase. The fact that this organism is already used in industry, is amenable to genetic manipulation, and has been demonstrated to product high titers of a single enzyme makes it a good choice to develop into a host for heterologous enzyme production. To understand how amenable this fungi is to expressing heterologous IL-tolerant enzymes, thirty-two bacterial and fungal genes, encoding beta- glucosidases, cellobiohydrolases and endoglucanases were screened for expression in *A. niger*. To probe the functionality of the enzymes that were successfully expressed in *A. niger*, we used the Jsalsa (the JBEI suite for automated lignocellulosic saccharification) platform to obtain activity profiles with respect to temperature, pH and ionic liquid and compared them to profiles of the same enzymes expressed in *E.coli*. It was found that many of the enzymes that expressed well in *E. coli* also expressed well in *A. niger*, and the enzymes that were profiled on Jsalsa appear to be functionally equivalent. Finally, we used enzymes produced by *A. niger* to assemble an IL-tolerant cellulase cocktail that was able to hydrolyze IL-pretreated biomass.

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