

Engineering Morphology and Secretion to Enhance the Productivity of Fungal Fermentations

Steven Harris (PI)¹, Mark Marten (Co-PI)²

¹University of Nebraska – Lincoln, ²University of Maryland – Baltimore County

Abstract: Enzymes such as cellulases and amylases play an essential role in the production of biofuels through their ability to convert biomass into fermentable sugars. The primary sources of these enzymes are filamentous fungi such as *Trichoderma reesei* and *Aspergillus* species. At the present time, the costs of enzyme production represent a significant limitation to the development of cellulosic biofuels as an alternative energy source. One way to reduce these costs is to improve enzyme yields by maximizing their secretion from fungal hyphae. However, we currently do not have complete understanding of the mechanism and pathways that mediate fungal secretion. We reason that filamentous fungi possess novel routes for secretion that are unique because of their highly polarized mode of growth. Moreover, these routes would be missed by standard reverse genetic approaches that have been used to characterize fungal homologues of yeast proteins implicated in vesicle trafficking. Accordingly, we have implemented a classical genetic approach that exploits the intimate links between morphology and secretion in fungal hyphae to identify mutants with enhanced secretion. Our screen utilizes both the model fungus *A. nidulans* and the industrial workhorse *T. reesei*. We have identified and initiated the phenotypic characterization of a large number of mutants for each of these fungi. Here, we propose to leverage the advanced capabilities of the DOE JGI and EMSL facilities to add significant value to our set of mutants. In particular, we have selected the 25 mutants from each fungus with the strongest secretion phenotypes. In partnership with JGI and EMSL, we will; (i) sequence each mutant to identify the genome polymorphism(s) responsible for the phenotype, (ii) use quantitative proteomics to characterize the secretome for each of these mutants, and (iii) employ helium ion microscopy to examine the hyphal surface of each mutant. These efforts will result in a robust dataset that we would otherwise not be able to generate given our current resources. In addition to stimulating basic research, we anticipate that our data will draw the interest of academic, government, and industrial researchers who are attempting to optimize the expression and production of fungal enzymes. Ultimately, we envision the use of our genomic, proteomic, and microscopy data will facilitate the breeding of improved production strains, thereby helping to reduce input costs associated with the production of biofuels.