

Fungal Biotechnology: How can we improve enzyme production?

Morgann C. Reilly^{1*} (mcreilly@lbl.gov), Saori Amaike Campen¹, Jinxiang Zhang¹, Joonhoon Kim¹, Junko Yaegashi¹, Yvette Tran¹, James Kirby¹, Jeffrey M. Skerker², Wendy S. Schackwitz³, Joel Martin³, Jon M. Jacobs⁴, Chee-Hong Wong³, Blake A. Simmons¹, Scott E. Baker^{1,4}, John M. Gladden¹, and **Jon K. Magnuson¹**

¹Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, CA; ²University of California-Berkeley and Energy Biosciences Institute, Berkeley, CA; ³Joint Genome Institute, Lawrence Berkeley National Laboratory, Walnut Creek, CA; and ⁴Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA

Project Goals: Development of recombinant protein production platforms in fungi for the production of ionic liquid-tolerant lignocellulolytic enzymes at high titers.

Efficient and economical deconstruction of biomass is critical for the success of lignocellulosic biorefineries. Biomass pretreatment with ionic liquids (ILs) tackles this issue as it increases biomass saccharification efficiency at lower cellulase loadings. However, some ILs inhibit the activity of commercial cellulases and must first be removed from the biomass, a costly mitigation.

To overcome this issue, the Microbial Communities and Enzyme Optimization teams at JBEI have identified cellulases that can function in the presence of ILs. These enzymes were expressed in *Escherichia coli*, which is a suitable host for lab-scale enzyme characterization but not for industrial-scale production of enzymes.

Filamentous fungi have been widely utilized for enzyme production in industry and therefore the Fungal Biotechnology team has focused on developing *Aspergillus niger* as a heterologous enzyme production host. Several approaches have been utilized to increase enzyme production in the fungus: 1) reverse genetics, 2) forward genetics and mutagenesis, 3) genetics parts development, and 4) characterization of a wide variety of heterologously expressed enzymes. We are also expanding our efforts to include fungal lignin deconstruction and conversion into advanced bioproducts.

This work was part of the DOE Joint BioEnergy Institute (<http://www.jbei.org>) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.

The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

A portion of the research was performed using EMSL, a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory.