At **Pacific Northwest National Laboratory**, we are working to solve energy challenges through biotechnology.

Pacific Northwest
NATIONAL LABORATORY
*Proudly Operated by* Battelle *Since 1965*

# Fungal Biotechnology for Biomass Conversion

Pacific Northwest National Laboratory is a leader in developing processes to convert cellulose and hemicellulose, the most abundant renewable, natural resources in the world, into fuels and chemicals. Our approach takes advantage of the primary organisms in nature for breaking down biomass—fungi.

By applying principles of systems biology and synthetic biology to various robust industrial fungi, PNNL optimizes each fungus to perform a specific function in the processes required to turn plant biomass into usable products. We are:

- Taking pathways found in wild-type fungi and moving them into industrial fungi that are robust enough to live on complex feed stocks, require only simple nutrients, and can survive in low pH environments. These engineered industrial fungi produce greater quantities of desired compounds.
- Increasing the selectivity of these strains to produce desired compounds, such as lipids, polyketides, organic acids and enzymes.
- Improving the efficiency of how these strains use sugars to increase yields of desired compounds.

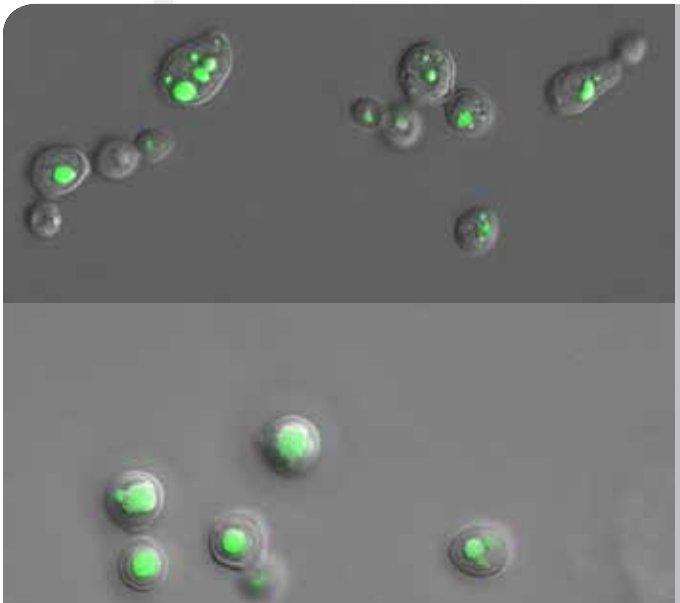
## EXPERTISE AND CAPABILITIES

As DOE's premier national laboratory with expertise in fungal systems, PNNL brings a multidisciplinary team of experts, who are drawing upon resources from across the laboratory, to the challenges of converting biomass to fuels and chemicals. Our unique package of staff expertise, instruments and capabilities includes:

- A decade of leadership in fungal biotechnology.
- Modern "omics" tools including genomics, proteomics, metabolomics, and transcriptomics to target genes for optimizing metabolic pathways.
- Metabolic modeling that supports engineering strains with increased sugar conversion efficiency and product selectivity.
- Bioreactors close the loop on PNNL's complete fungal bioprocess development—from gene discovery to a tried and verified process description.

Additionally, EMSL, the Environmental Molecular Sciences Laboratory, offers mass spectrometry for metabolomics, proteomics and lipidomics, microscopy for fine structure visualization and studies of microbial systems, and nuclear magnetic resonance (NMR) spectroscopy for fermentation product identification. EMSL is a national Department of Energy user facility at PNNL.

impacts



## IMPROVING THE PRODUCTION OF BIOFUEL PRECURSORS

PNNL is applying its expertise in systems biology and advanced imaging to correlate gene expression and lipid production in the yeast (single-celled fungus) *Lipomyces starkeyi* to rapidly identify targets for genetic manipulation. The images from a confocal microscope at the left show the yeast at one day (upper) and two days (lower) of growth. The bright green circles are actual images of the rapidly expanding lipid bodies within the yeast cells (larger grey ovals). Engineering a strain of *Lipomyces starkeyi* that can produce greater quantities of lipids faster will improve the cost-effectiveness of producing biofuel precursors.

## DELIVERING IMPACT

PNNL has led a team of international scientists working with DOE's Joint Genome Institute (JGI) to sequence and analyze the genome of *Aspergillus niger*, an industrial workhorse fungus that has been used on a commercial scale for decades for production of citric acid and enzymes deployed in breaking down plant cell walls to sugars. Similarly, PNNL collaborated on JGI research to sequence the genome of *Trichoderma reesei*, another key producer of industrial enzymes that are used to break down biomass to sugars for improved biofuel production.

As a result of our expertise in fungal research, PNNL is leading the Joint BioEnergy Institute's (JBEI's) efforts to engineer a strain of *Aspergillus niger* from the ground up to produce high concentrations of novel variants of enzymes that break down biomass to sugars at higher temperature and salt concentrations.

PNNL also is partnering with the University of Southern California, the University of Kansas, the State University of New York at Buffalo, and the Technical University of Denmark to identify and target specific naturally occurring fungal metabolites with the most potential for making fuels and chemicals and then manipulate the pathways to increase selectivity and productivity.

**John Holladay**
(509) 375-2025
john.holladay@pnnl.gov

**Rick Orth**
(509) 375-6709
rick.orth@pnnl.gov

**Chemical and Biological Process Development**
Pacific Northwest National Laboratory
P.O. Box 999, MSIN P8-60
Richland, WA 99352

## ABOUT BSEL

The Bioproducts, Sciences, and Engineering Laboratory (BSEL) is a multipurpose R&D facility that includes:

- Modern laboratories for fungal strain and bioprocess development and optimization
- 2,500-square-foot high-bay biorefinery for technology scale up
- Analytical chemistry lab equipped with a wide array of analytical instruments.

## COLLABORATIONS

PNNL collaborates with multiple industrial partners to keep its research relevant to the needs of industry and to facilitate technology transfer to the public domain. We also have major leadership roles in the Joint BioEnergy Institute (JBEI), DOE's National Advanced Biofuels Consortium (NABC) and National Alliance for Advanced Biofuels (NAABB). We partner with world-class regional, national and international universities and have active collaborations with other national laboratories.

## ABOUT EMSL

EMSL, the Environmental Molecular Sciences Laboratory, is a U.S. Department of Energy national scientific user facility located at Pacific Northwest National Laboratory. EMSL provides integrated experimental and computational resources for discovery and technological innovations. To learn more about the science conducted at EMSL as well as the instruments and expertise available to users, visit ***http://www.emsl.pnnl.gov/***.

## ABOUT PNNL

PNNL's biomass conversion work is an important element in a wider portfolio of research and development funded by the U.S. Department of Energy, other federal agencies and the private sector. PNNL is a DOE Office of Science National Laboratory where interdisciplinary teams advance science and technology and deliver solutions to America's most intractable problems in energy, national security and the environment. To learn more about PNNL, visit ***http://www.pnnl.gov/***.

Pacific Northwest NATIONAL LABORATORY

*Proudly Operated by* Battelle *Since 1965*

U.S. DEPARTMENT OF ENERGY