5 February 2018

**Chemical genomic-guided engineering of γ-valerolactone (GVL)-tolerant yeast**

Engineering yeast tolerance to a promising biomass deconstruction solvent

**The Science**

In this study, we used chemical genomics (CG) to identify the mechanisms of GVL toxicity to fermentative microbes. We identified gene deletions that confer sensitivity or tolerance to the solvent and then used this knowledge to engineer a xylose-fermenting yeast strain with improved tolerance to GVL and enhanced conversion of sugars to biofuel.

**The Impact**

Solvents such as GVL are promising for use in deconstructing biomass, but can adversely impact the microbes used for fermentation. Here, chemical genomic-guided engineering provided a rapid method for tailoring existing yeast strains to specific chemical stressors, including a GVL-tolerant yeast strain with increased conversion efficiency.

**Summary**

Biomass deconstruction using the solvent γ-valerolactone (GVL) has several advantages over more traditional deconstruction methods; however biological conversion to biofuels can be challenging as fermentation microbes are sensitive to residual solvent. Researchers at Great Lakes Bioenergy Research Center sought to identify the mechanisms of GVL toxicity using chemical genomics (CG), which measures the impact of small molecules on microbes using bar-coded deletion or knockout collections of non-essential genes. CG profiling of GVL predicted that this chemical affects *Saccharomyces cerevisiae* membranes and membrane-bound processes. Indeed, permeability assays showed that GVL compromised *S. cerevisiae* cell membrane integrity and interacted synergistically with ethanol. CG profiling also revealed that deletion of the functionally related enzymes Pad1p and Fdc1p, which act together to decarboxylate cinnamic acid and its derivatives to vinyl forms, increases yeast tolerance to GVL. Moreover, deletion of *PAD1* and *FDC1* in a xylose-fermenting yeast strain led to improved growth, sugar utilization, and ethanol production in synthetic hydrolysate containing 1.5% GVL relative to the non-engineered strain. Chemical proteomic profiling of the engineered strain revealed that enzymes involved in ergosterol biosynthesis were more abundant in the presence of GVL and cellular levels of this sterol were elevated compared to the background strain. These results suggest that one route to GVL tolerance in yeast is through alteration of membrane fluidity, which is heavily influenced by ergosterol levels. Future studies are needed to address the role of *PAD1* and *FDC1* in ergosterol biosynthesis. This study also illustrates the utility of chemical genomics approaches to rapidly identify cellular targets of small molecules and strategies to engineer microbial strains for improved fermentative performance.

**Contacts (BER PM)**

N. Kent Peters
Program Manager, Office of Biological and Environmental Research
kent.peters@science.doe.gov, 301-903-5549

**(PI Contact)**

Jeff Piotrowski
Yumanity Therapeutics
jpiotrowski@yumanity.com

Robert Landick

University of Wisconsin-Madison
landick@bact.wisc.edu

**Funding**

This work was funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494). Additional funding was provided by grants from the National Institutes of Health and the National Science Foundation.

**Publications**

Bottoms, S. et al. “Chemical genomic guided engineering of gamma-valerolactone tolerant yeast.” *Microbial Cell Factories* **17**, 5 (2018), DOI:10.1186/s12934-017-0848-9.

**Related Links**

 <https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-017-0848-9>

**PM Recommendation for SC Web Publication**