

## Producing Valuable Bioproducts from the Waste Residue of Biofuel Production

John F. Wolters<sup>1,2,\*</sup> (jwolters@wisc.edu), Dana Opulente<sup>1,2</sup>, Jacek Kominek<sup>1,2</sup>, Rachel M Schneider<sup>1,2</sup>,  
Chris Todd Hittinger<sup>1,2</sup>

<sup>1</sup> DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI;

<sup>2</sup> Laboratory of Genetics, Genome Center of Wisconsin, Wisconsin Energy Institute, J.F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, WI

**Project Goals: The Great Lakes Bioenergy Research Center is developing the production of sustainable biofuels and bioproducts from dedicated energy crops grown on marginal lands. Our research is focused on identifying non-model yeasts that are capable of utilizing the residual carbon that is not metabolized by the primary fermentation of ethanol from cellulosic feedstocks. For species that are capable of growth using the waste residue from cellulosic ethanol production we will identify valuable bioproducts produced, such as lipids that can be used for biodiesels or as substitutes for other oils. Incorporating the conversion of the waste residue into these products will increase the cost efficiency of the biofuel production pipeline.**

During cellulosic ethanol production cellulosic feedstocks are hydrolyzed to produce sugar streams which are fermented by a primary microorganism to produce ethanol. Some microbes, such as engineered strains of the yeast *Saccharomyces cerevisiae* or the anaerobic bacterium *Zymomonas mobilis*, are capable of metabolizing multiple sugars present in hydrolysate including xylose. However, even after consumption of glucose and xylose a large fraction of the carbon from the original feedstocks remains in the waste residue after ethanol distillation. The use of non-model microbes that can metabolize the complex and uncharacterized compounds remaining in the waste residue could turn this waste product into a valuable feedstock for production of additional bioproducts.

The fungal subphylum *Saccharomycotina* contains over one thousand budding yeast species including many with metabolic capacities not present in model species such as *S. cerevisiae*. New genomics resources being built for these species can enable predictions regarding which species may be able to metabolize the complex substrates found in the waste residue<sup>1</sup>. We demonstrate that the ability to grow on the waste residue is widely distributed across yeast species. The most robust growth profiles are found among basal lineages which include biotechnologically important species including *Yarrowia lipolytica* and *Lipomyces starkeyi* known for their capacity to produce and store high levels of intracellular lipid. We show that lipid production triggered by nitrogen limitation is variable among these species and demonstrate considerable lipogenesis phenotypes in several species capable of robust growth on the waste residue. We consider engineering strategies to better understand the triggers of lipogenesis and how to achieve high lipid titres during growth on the waste residue.

### References

1. Shen, X.-X. *et al.* Tempo and Mode of Genome Evolution in the Budding Yeast Subphylum. *Cell* **175**, 1533–1545 (2018).

*This material is based on work supported by the Great Lakes Bioenergy Research Center, U.S., Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494*