Engineering and optimization of lignin catabolic pathways in *Rhodosporidium toruloides*

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https://www.jbei.org/research/deconstruction/fungal-biotechnology/

Project Goals:

The vision of JBEI is that bioenergy crops can be converted into economically-viable, carbon-neutral, biofuels and renewable chemicals currently derived from petroleum, and many other bioproducts that cannot be efficiently produced from petroleum. JBEI's mission is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. When fully scaled, JBEI's technologies will enable the production of replacements for petroleum derived gasoline, diesel, jet fuel, and bioproducts. In doing so, JBEI will reduce the nation's dependence on fossil fuels, significantly reduce the amount of carbon added to the atmosphere, reduce contamination of the environment, and provide the scientific tools and knowledge required to transform the bioenergy marketplace.

Abstract:

The oleaginous basidiomycete, *Rhodosporidium toruloides* (also known as *Rhodotorula toruloides*), is rapidly gaining traction as an industrial host for converting lignocellulosic biomass into value-added products. The yeast is naturally capable of co-utilizing multiple carbon sources such as pentoses, hexoses, and lignin-derived aromatics. It also naturally accumulates substantial amounts of acetyl-CoA and malonyl-CoA, the building blocks for many desirable biochemicals, within its cytosol. Many labs within the Department of Energy and academia have leveraged these properties to use *R. toruloides* to valorize biomass into compounds such as bisabolene.

However, these efforts have largely focused on utilization of pentose and hexose feedstocks, while neglecting the carbon in biomass that is contained within aromatic compounds. To fully utilize the carbon available in lignocellulosic feedstocks, efforts must be directed to valorize these aromatics. Here, we describe our efforts with the Joint BioEnergy Institute to do so. We focus primarily on the aromatic compound *p*-coumarate, as the metabolic pathway to this compound in *R. torulolides* is well-established in through the enzymes phenylalanine ammonia lyase and 4-coumarate-CoA ligase.

We first build upon our metabolic model of *p*-coumarate degradation in *R. toruloides* by employing CRISPR editing to delete key steps within the pathway. We show that this leads to substantial accumulation of key beachheads, protocatechuate (PCA, 6.7 ± 2.2 g/L) and 4-

hydroxybenzoate (4HBA, 4.6 ± 0.1 g/L) in minimal media. We also show considerable buildup of 4HBA (1.3 ± 0.1 g/L) during fermentation of one of these strains in hydrolysates derived from lignocellulosic biomass. We next describe our attempts to integrate four heterologous pathways for production of curcuminoids, naringenin, resveratrol, and 2-pyrone-4,6-dicarboxylic acid (PDC). We obtain the most promising results with the later compound, observing signs of its precursor compound 4-carboxy-2-hydroxymuconate-6-semialdehyde (CHMS) as well as 0.4 g/L of PDC itself. Finally, we describe our efforts to adapt *R. toruloides* to better utilize *p*-coumarate by employing Tolerance Adaptive Laboratory Evolution (TALE), generating an evolved strain that robustly grows in media with 20 g/L *p*-coumarate as the sole carbon source. Taken together, these results describe how the Joint BioEnergy Institute has sought to maximize the productive potential of *R. toruloides* as an industrial chassis for fully valorizing the carbon in lignocellulosic biomass.

This material is based upon work supported by the U.S. Department of Energy (DOE), Office of Science. Work conducted at the DOE Joint BioEnergy Institute was supported by the U.S. DOE, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02- 05CH11231