Rhodosporidium toruloides for conversion of depolymerized cellulose, hemicellulose, and lignin into bioproducts

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Project Goals: Conversion of all major components of lignocellulose into advanced bioproducts has been a long elusive goal that is essential for enabling a robust bioeconomy. This project, performed in the Fungal Biotechnology group in the Deconstruction Division at JBEI, seeks to establish and utilize *Rhodosporidium toruloides* as a platform organism that can convert depolymerization products of cellulose, hemicellulose and lignin into biofuels and bioproducts.

Economical conversion of lignocellulosic biomass to biofuels and bioproducts is central to the establishment of a robust bioeconomy. Efficient conversion of lignocellulose requires the utilization of all its primary components (cellulose, hemicellulose, and lignin), yet no microbe in commercial use today can achieve this feat. To that end, we explored the utility of *Rhodosporidium toruloides* as a new platform organism for the production of biofuels and bioproducts from deconstructed plant biomass. In this study, *Rhodosporidium toruloides* was engineered to produce two non-native terpenes with biofuel (bisabolene) and pharmaceutical (amorphadiene) applications from a mixture of depolymerized cellulose (glucose), hemicellulose (xylose), and lignin (*p*-coumaric acid). The titers of these bioproducts in synthetic defined media with 2% glucose were 294mg/L and 36g/L, respectively. It was also cultivated on corn stover hydrolysates prepared by two different pretreatment methods, including one using the novel biocompatible ionic liquid choline α-ketoglutarate. This organism was able to tolerate the ionic liquid that was used for pretreatment and was also able to utilize all of the three major carbon sources in the hydrolysate to support growth and bisabolene production. Finally, we demonstrate that this organism is amenable to high-gravity fed-batch fermentation, reaching a titer of 680mg/L in alkaline corn stover hydrolysate. This study establishes *R. toruloides* as a new platform for the simultaneous conversion of depolymerized cellulose, hemicellulose, and lignin into biofuels and bioproducts.

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