

## Engineering *Rhodospiridium toruloides* for Bioproduction of Polyketide Synthase and p-Coumarate Derived Compounds

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### Project Goals/Abstract:

The oleaginous yeast *Rhodospiridium toruloides* is an ideal chassis for the valorization of lignocellulosic biomass into value-added products due to its natural capacity to co-utilize multiple carbon sources, and its ample pool of malonyl-CoA. Here we leverage this advantage by incorporating seven heterologous pathways to convert p-coumarate and/or malonyl-CoA into useful bioproducts. Pathways for two polyketides (6-methylsalicylic acid (6MSA) and triacetic acid lactone (TAL)) were introduced. While no 6MSA production was observed, a substantial amount ( $1.65 \pm 0.06$  g/L) of TAL was secreted in standard media without any optimization, leaving much room for improvement on these already significant titers. Five other pathways for converting p-coumarate, a lignin-derived precursor, were also introduced for biosynthesis of naringenin, resveratrol, curcuminoids, 4-hydroxybenzoate (4HBA), and 2-pyrone-4,6-dicarboxylic acid (PDC). We show indications of three of these pathways working, with both the production and subsequent consumption of resveratrol, conversion of protocatechuate into a toxic aldehyde intermediate (4-carboxy-2-hydroxymuconate-6-semialdehyde) between it and PDC, and a remodeling of the p-Coumarate consumption pathway resulting in extracellular accumulation of  $3.17 \pm 0.10$  g/L 4HBA. Finally, we prelude the use of Tolerance Adaptive Laboratory Evolution (TALE) to enable robust growth of *R. toruloides* in 20 g/L p-Coumarate. These new results broaden the already substantial spectrum of biofuels and bioproducts that *R. toruloides* has been shown to produce.

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