CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Metabolic Engineering of Oleaginous Yeast *Rhodotorula toruloides* for Overproduction of Triacetic Acid Lactone

Background/Objective

Rhodotorula toruloides is a promising production host to synthesize chemicals from biomass thanks to its ability to grow on lignocellulosic biomass and its high production of acetyl-CoA, a precursor molecule to valuable chemicals such as triacetic acid lactone (TAL). TAL can be converted to a variety of valuable intermediates and end products such as phloroglucinol, acetylacetone, and sorbic acid. The goal of this work is to establish *R. toruloides* as a platform organism for high production of TAL through metabolic engineering and fed-batch bioreactor fermentation.

Approach

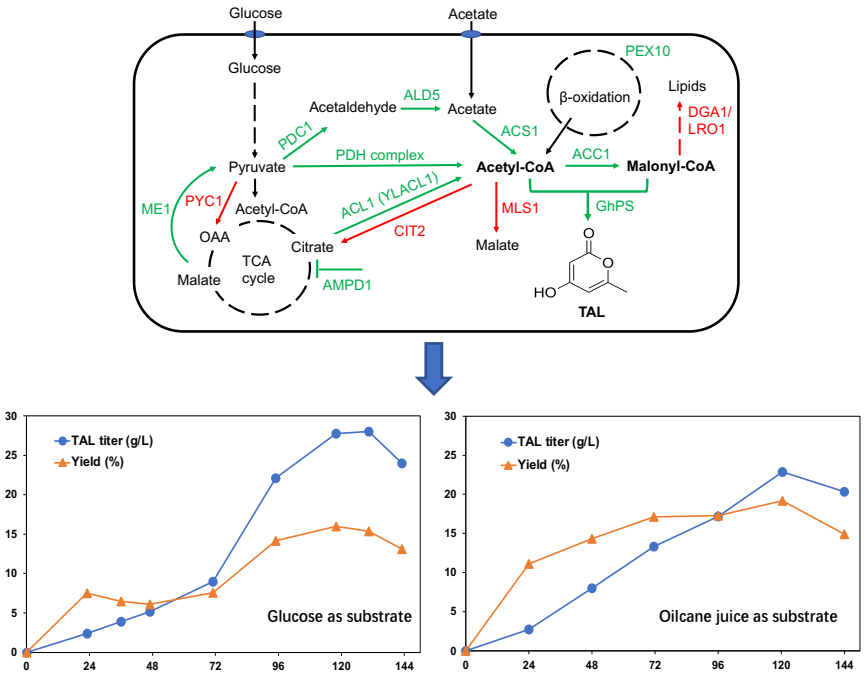
- Screened several sources of the 2-pyrone synthase (2PS) gene, and functionally expressed the gene from *Gerbera hybrida* in *R. toruloides* to produce TAL.
- Systematically evaluated various metabolic targets, such as ATP-citrate lyase (ACL1) and acetyl-CoA carboxylase (ACC1), to increase acetyl-CoA and malonyl-CoA levels, which increase TAL production.
- Performed fed-batch bioreactor fermentation using glucose or oilcane juice as substrates and evaluated TAL production.

Results

- *R. toruloides* with 2PS from *G. hybrida* produced 2 g/L TAL in a culture tube.
- Overexpression of metabolic target ACL1 improved TAL production by 45%, and additional overexpression of ACC1 further increased TAL by 29%.
- *R. toruloides* engineered with 2PS and the overexpressed enzymes produced 28 g/L or 23 g/L TAL in glucose or oilcane juice medium using fed-batch fermentation, respectively.

Significance/Impacts

This work demonstrates that *R. toruloides* is a promising host to produce TAL and other acetyl-CoA-derived chemicals from renewable biomass.



Metabolic engineering strategies and fed-batch bioreactor fermentation of *R. toruloides* for TAL production

Cao, M., Tran, V., Qin, J., Olson, A., Mishra, S., Schultz, J.C., Huang, C., Xie, D., Zhao, H. June 14, 2022. "Metabolic Engineering of Oleaginous Yeast *Rhodotorula toruloides* for Overproduction of Triacetic Acid Lactone." Biotechnology and Bioengineering 2022:1-12. DOI: 10.1002/bit.28159.