BRC Science Highlight
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Development of a CRISPR/Cas9 System for High-Efficiency Multiplexed Gene Deletion in *Rhodosporidium toruloides*

Background/objective

Rhodosporidium toruloides is a promising platform organism for producing value-added bioproducts due to its capacity to grow on lignocellulosic sugars. However, research in this area is hindered by a lack of tools for genetic manipulation of this yeast strain. Here, researchers developed a CRISPR/Cas9 system for gene knockout in *R. toruloides*.

Approach

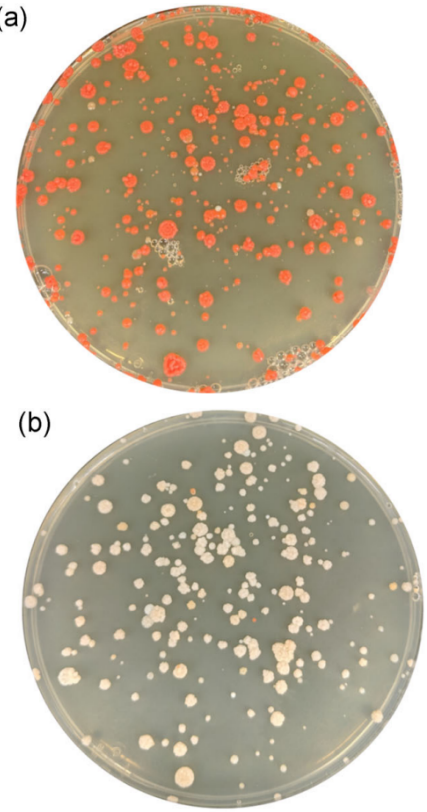
- Iteratively developed methods for expressing both gRNA and Cas9 in *R. toruloides* NP11.
- Tested methods on genes in the beta-carotene biosynthetic pathway (*CRTYB* and *CRTI*), and on the auxotrophic selection marker *LEU2.*
- Verified capacity of engineered system to achieve both single- and double-gene knockouts.

Results

- A greater than 95% knockout rate was achieved for various single-gene targets.
- Double-gene knockout mutants were achieved with an efficiency of 78%.

Significance

- This is the first CRISPR/Cas9 system for modular, targeted gene knockout in *R. toruloides*.
- This tool can be used to accelerate future metabolic engineering of this promising non-model yeast.



gRNA expression driven by a 5S-tRNA fusion promoter (b) yielded higher knockout rate of the *CRTYB* gene, as indicated by albino colonies, than gRNA expression driven by 5S rRNA (a).

Schultz, J., et al. 2019. "Development of a CRISPR/Cas9 System for High Efficiency Multiplexed Gene Deletion in Rhodosporidium toruloides*." **Biotechnology and Bioengineering.** 1-7. DOI:10.1002/bit.27001*

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