BRC Science Highlight
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Domesticating a Food Spoilage Yeast into an Organic Acid-Tolerant Metabolic Engineering Host: Lactic Acid Production by Engineered *Zygosaccharomyces bailii*

Background/objective

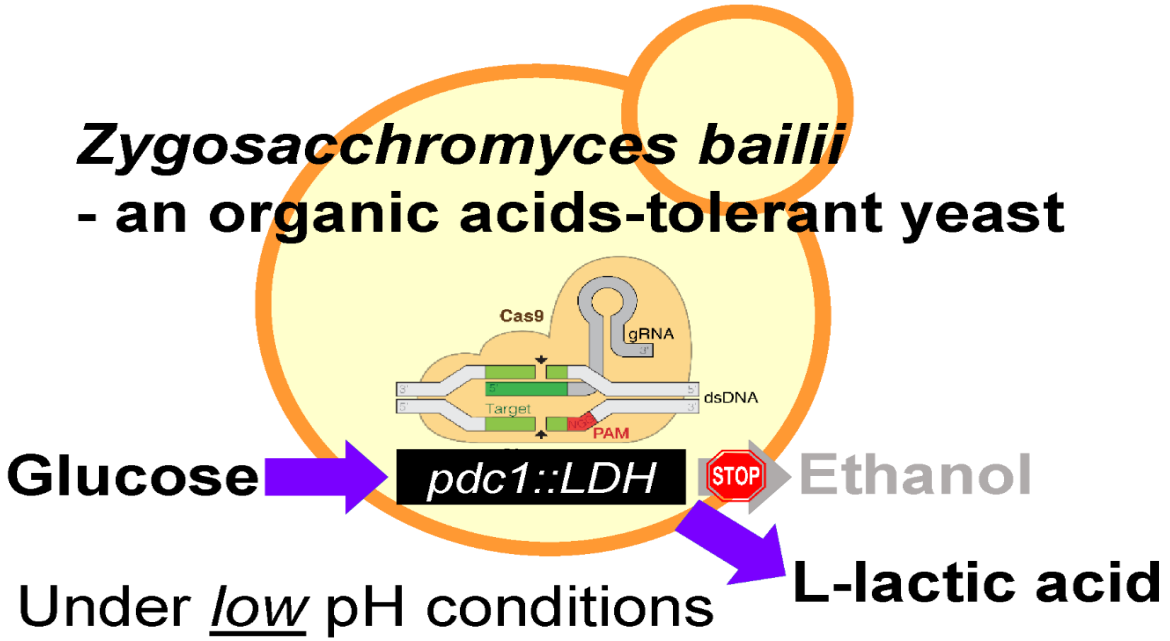
Non-conventional yeasts are getting more attention as an alternative microbial cell factory for organic acid production. In this study, multiple strains of *Zygosaccharomyces bailii*, a food spoilage yeast that can grow under the presence of organic acids, were screened with the goal of finding an appropriate metabolic engineering host for producing lactic acid.

Approach

- In collaboration with USDA-ARS (Peoria, Ill.), acquired and screened different *Z. bailii* strains for lactic and acetic acid tolerance at low pH, and selected the most robust tolerance to organic acids (*Z. bailii* Y7239).
- Adopted CRISPR-Cas9 system for *Z. bailii* usage and disrupted *ZbADE2* gene as a proof of functionality.
- Simultaneously deleted major pyruvate decarboxylase (*PDC*) genes and integrated lactate dehydrogenase gene (*LDH*) to achieve homofermentative lactic acid production in *Z. bailii* Y7239.

Results

- Demonstrated applicability of CRISPR-Cas9 system for *Z. bailii* genome engineering by disrupting *ZbADE2* gene.
- Created a *Z. bailii* strain that could produce 35g/L lactic acid without production of ethanol by disrupting major *PDC* genes and integrating *LDH*.



Graphical representation of metabolic engineering of *Z. bailii* using CRISPR-Cas9 system

Significance

The results showed the feasibility of using the CRISPR-Cas9 in *Z. bailii* for future engineering for organic acid production.

*Kuanyshev, N., et al. 2020. "Domesticating a Food Spoilage Yeast into an Organic Acid-Tolerant Metabolic Engineering Host: Lactic Acid Production by Engineered Zygosaccharomyces bailii." **Biotechnology and Bioengineering**, 2020, 1-11, DOI: 10.1002/bit.27576.*

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