

Overexpression of *RCK1* Improves Acetic Acid Tolerance in *Saccharomyces cerevisiae*

Background/objective

While lignocellulosic biomass has the potential to yield abundant sugars for conversion to biofuels, the depolymerization process also generates high concentrations of fermentation inhibitors such as acetic acid, which are toxic to industrially important yeasts. In this study, researchers used inverse metabolic engineering to identify and test the mechanism by which gene *RCK1* improved acid tolerance in *Saccharomyces cerevisiae*.

Approach

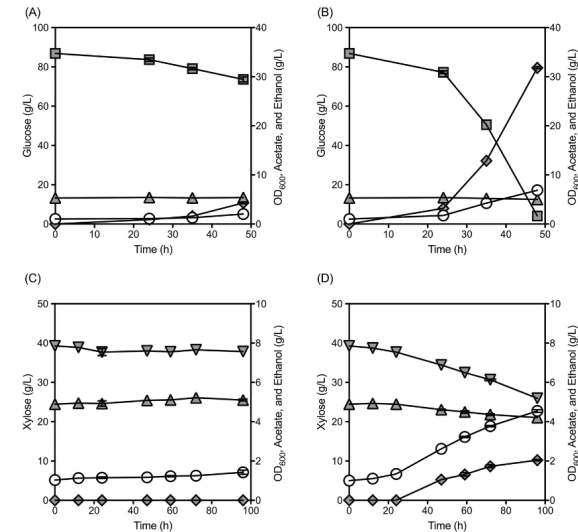
- ❖ A yeast genomic library was screened in *S. cerevisiae* under toxic levels of acetic acid, and the transformant with the plasmid containing the gene *RCK1* was identified as being able to grow under acetic acid stress.
- ❖ *RCK1*-overexpressing multi-copy plasmids were transformed into *S. cerevisiae*; glucose and xylose fermentation were tested against an empty plasmid control.
- ❖ Intracellular levels of reactive oxygen species (ROS) were compared between *RCK-1* overexpressing strains and a control.

Results

- ❖ The overexpression of *RCK1* in engineered *S. cerevisiae* enhanced acetic acid tolerance under both glucose- and xylose-fermenting conditions.
- ❖ Experimental results indicate that *RCK1* attenuates acetic acid stress by reducing intracellular levels of ROS.

Significance

- ❖ This is the first demonstration that *RCK1* overexpression improves acetic acid tolerance in *S. cerevisiae*. The inverse metabolic engineering approach used here may be useful in future efforts to improve yeast tolerance to other fermentation inhibitors such as hydroxymethylfurfural and furfural.



***RCK1*-overexpressing plasmid (B,D) enhances glucose and xylose consumption in *S. cerevisiae* in the presence of toxic acetic acid levels as compared to empty plasmids (A,C). Symbols: cell growth (○), glucose (□), xylose (▼), acetic acid (▲), ethanol (◇)**