BRC Science Highlight
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A Genetic Toolbox for Metabolic Engineering of *Issatchenkia orientalis*

Background/objective

The non-model yeast *Issatchenkia orientalis* is a promising candidate for production of organic acids due to its ability to grow at low pH. However, progress has been hindered by a lack of effective metabolic engineering tools. The work reported here describes three new tools for genetic manipulation in *I. orientalis*, adding to two existing tools that were previously published by this research group.

Approach

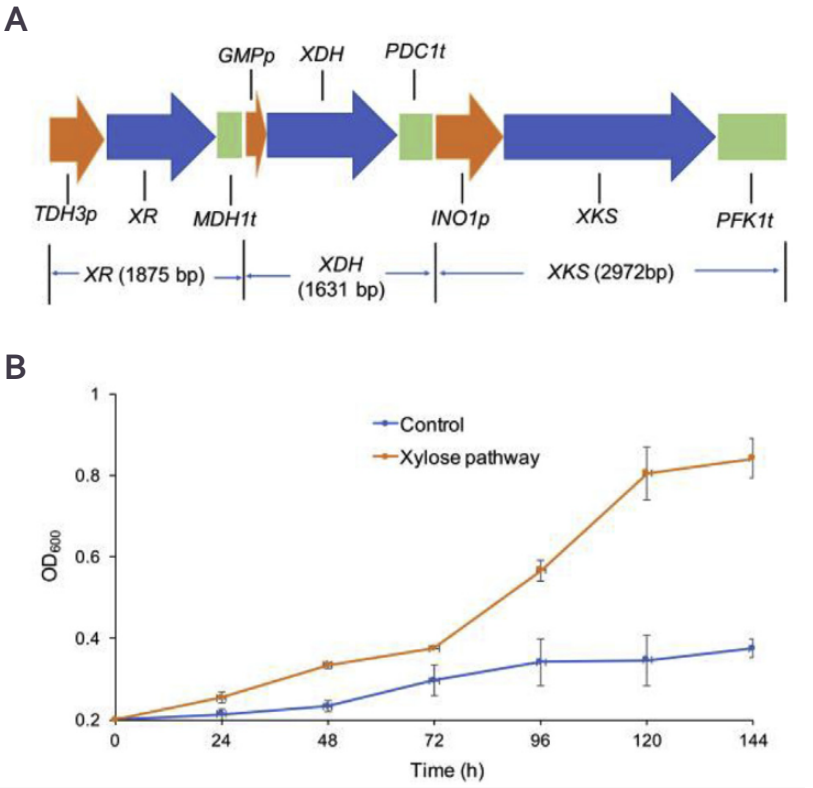
- Bioinformatics and functional screening were used to identify a centromere-like sequence from the *I. orientalis* genome.
- RNA-seq analysis and a fluorescence reporter were used to identify constitutive promoters and terminators.
- Different sizes and numbers of DNA fragments were used to characterize *in vivo* DNA assembly.

Results

- Incorporation of the 0.8 kb centromere-like sequence improved plasmid stability.
- A set of constitutive promoters and terminators were discovered and characterized under varying culture conditions.
- An efficient *in vivo* assembly method was developed for plasmid assembly and used to construct a xylose-utilization pathway in *I. orientalis*.

Significance

The addition of these three tools rounds out a comprehensive toolkit for genetic manipulation in *I. orientalis*, which will facilitate the metabolic engineering of this yeast strain for production of organic acids and other valuable bioproducts.



The new tools were used to construct a xylose utilization pathway in *I. orientalis* (A), which resulted in improved growth on xylose (B).

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