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Metabolic Engineering of Low-pH-Tolerant Non-Model Yeast, *Issatchenkia orientalis*, for Production of Citramalate

Background/Objective

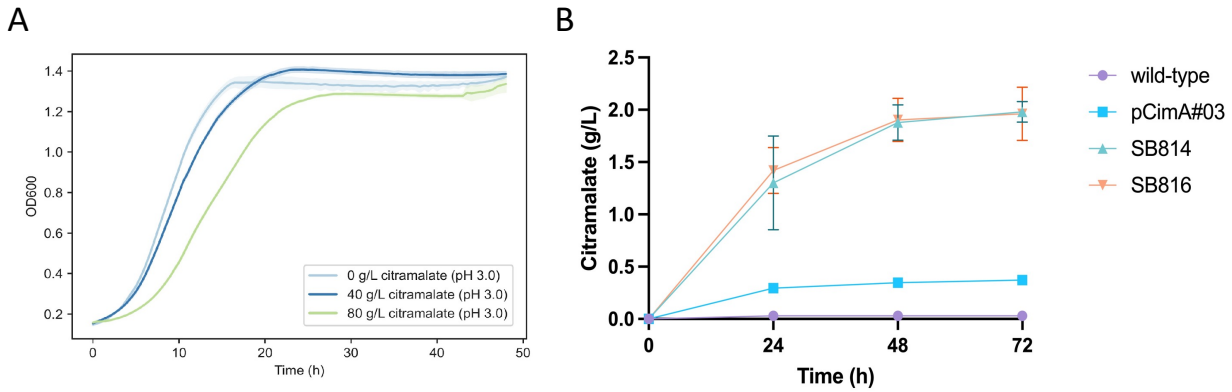
Manufacturing methyl methacrylate (MMA), an important petrochemical, generates a significant environmental footprint. To address this issue, combined biological and chemical synthesis, also known as semisynthesis, may be a promising alternative to reduce cost and environmental impact. This process involves biosynthesizing citramalate, followed by a base-catalyzed decarboxylation and dehydration to produce MMA. To successfully implement this semisynthesis process, it is crucial to have a yeast strain that can produce the MMA precursor citramalate in a low-pH environment. We selected a low-pH-tolerant non-model yeast, *Issatchenkia orientalis*, as a host platform for producing citramalate.

Approach

Using sequence similarity network analysis and subsequent DNA synthesis, an active citramalate synthase gene (*Methanocaldococcus jannaschii cimA*) for expression in *I. orientalis* was selected. A PiggyBac transposon system for *I. orientalis* was adapted, allowing for the effects of different *cimA* gene copy numbers and integration locations to be explored simultaneously.

Results

The genome-integrated-*cimA* strains produced 2.0 g/L citramalate in 48 hours and a yield of up to 7% mol citramalate/mol consumed glucose.



(A) *I. orientalis* could tolerate 80 g/L of citramalate at pH 3.0. (B) The genome-integrated-cimA strains, *I. orientalis* SB814 and SB816, produced the most citramalate, with a titer of 2.0 g/L, which is 6-fold higher than their plasmid counterpart.

Significance/Impacts

- *I. orientalis* shows remarkable tolerance to industrially relevant stresses (low pH) for citramalate production.
- The PiggyBac transposon system offers a rapid exploration of integration sites and copy numbers, leading to high-production strains.

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