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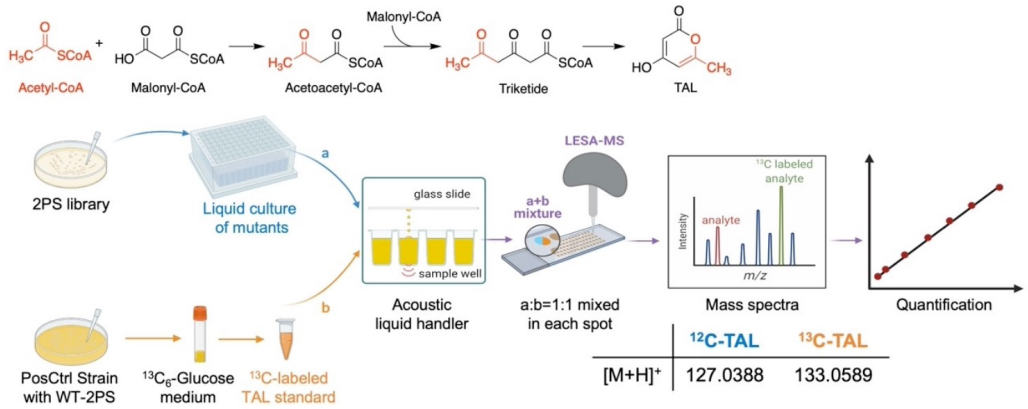
Enhancing 2-Pyrone Synthase Efficiency by High-Throughput Mass-Spectrometric Quantification and In Vitro/In Vivo Catalytic Performance Correlation

Background/Objective

Engineering efficient biocatalysts is essential for metabolic engineering to produce valuable bioproducts from renewable resources. However, the complexity of cellular metabolic networks makes it challenging to translate successful in vitro performance to cellular environments. To meet such a challenge, accurate and efficient quantification methods are necessary to screen large sets of mutants from complex cell cultures that also correlate catalytic parameters *in vitro* with their performance in cells. Here, we employed a mass-spectrometry (MS) based high-throughput quantitative method to screen new mutants of 2-pyrone synthase (2PS) for triacetic acid lactone (TAL) biosynthesis.

Approach

A ^{13}C-labeled TAL sample was prepared by feeding *E. coli* containing wild type 2PS with ^{13}C-labeled glucose, which was utilized as an internal standard. A liquid culture containing the internal standard was mixed equally with liquid cultures of 2PS mutants onto a glass slide using an acoustic liquid handler. This mixture droplet was dried, automatically extracted with solvents by liquid extraction surface analysis (LESA) and injected into MS for quantification. Mutants of interest were studied using enzymatic kinetic assays and synthetic biology tools to determine how 2PS evolved in the directed evolution process.



Graphical summary of the TAL conversion, biosynthesis and mass-spectrometry based quantification method: the 2PS catalyzed TAL biosynthetic pathway and the LESA-MS quantification workflow.

Results

We discovered two new mutants with the highest improvement in titer (46-fold) and the fastest k_{cat} (44-fold) over the wild type 2PS, respectively. Analysis revealed that a fast reaction rate under limiting intracellular substrate concentrations is important for in-cell biocatalysis.

Significance/Impacts

The demonstrated workflow combines protein engineering with high-throughput screening, and the insights gained from the correlation between in-vitro and in-cell catalytic properties are highly applicable to other bioproducts.

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