BRC Science Highlight
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A Mass Spectrometry-Based High-Throughput Screening Method for Engineering Fatty Acid Synthases with Improved Production of Medium Chain Fatty Acids

Background/objective

Microbially produced free fatty acids (FFAs) may provide a sustainable alternative to petroleum for production of value-added chemicals. However, the physiochemical properties of FFAs, determined by their chemical structures, remain challenging to control via enzymatic and metabolic engineering. A roadblock to using high-throughput (HT) methods to address these challenges has been a lack of corresponding HT analytical methods for medium chain fatty acid (MCFA) characterization. Here, researchers address this challenge by demonstrating a novel, separation-free mass spectroscopy (MS)-based HT screening method for MCFA analysis using membrane lipids as a proxy.

Approach

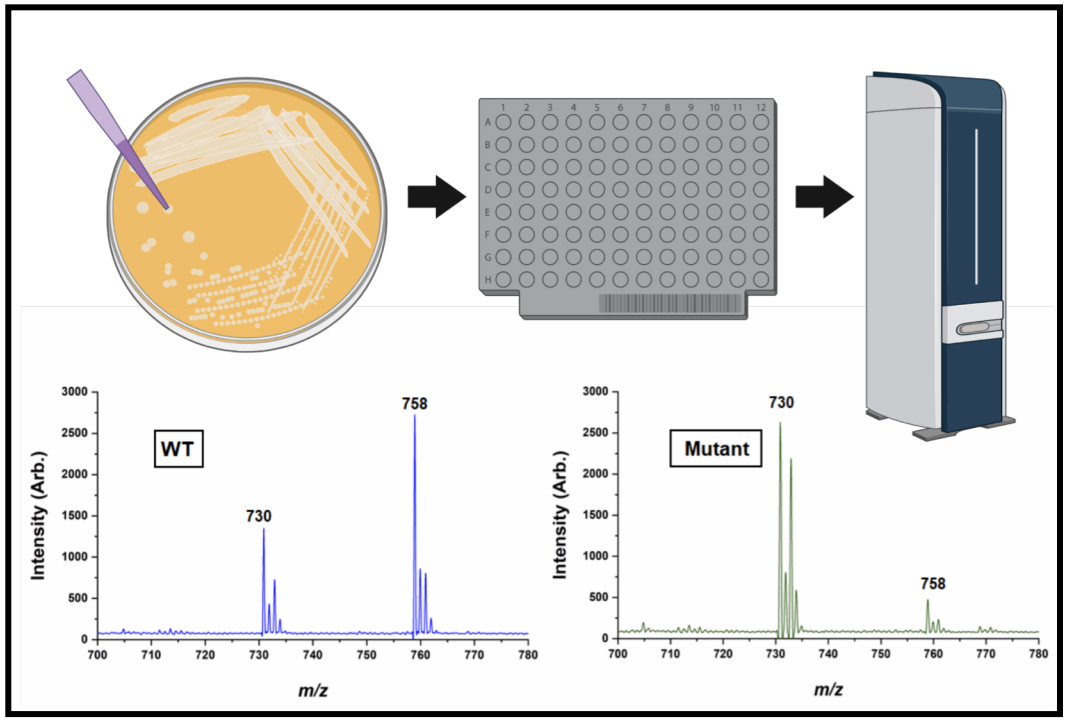
- Developed a matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) MS-based method for membrane phosphatidylcholine (PC) detection in yeast colonies and tested utility of PC as an indication for improved MCFA production.
- Used site-saturation mutagenesis in combination with the new MS-based method to *de novo* synthesize and characterize *Saccharomyces cerevisiae* FAS2 mutants with improved MCFA production.

Results

- Efficiently analyzed membrane lipids at a rate of ~2 second per sample.
- Confirmed positive correlation between short acyl-chain PC and MCFA levels.
- Identified *S. cerevisiae* mutants with increased C6 (20-fold), C8 (400-fold), C10 (16-fold) and C12 (16-fold) FA content when compared to the wild type.

Significance

This work presents a novel, high-throughput, separation-free method for characterizing MCFA production in yeast. This tool will expand analytical capacity for high-throughput metabolic engineering studies to facilitate production of these industrially important bioproducts.



Workflow of MALDI-ToF MS-based high-throughput screening method (top). WT: *m/z* 730/758 < 1; Mutant: *m/z* 730/758 > 1 correlates to increased MCFA production (bottom).

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