Evaluation of Strategies to Narrow the Product Chain-Length Distribution of Microbially Synthesized Free Fatty Acids

CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Background/Objective

To enter the oleochemical market with improvements over the incumbent technology, microbial platforms must demonstrate selective production of medium-chain oleochemicals, produce industrial-scale product titers with a small land-use requirement, and utilize low-cost, low-footprint feedstocks. Focusing on developing selectivity, the dominant strategy to tailor the chain-length distribution of free fatty acids (FFA) synthesized by microbial or plant hosts is expressing a selective acyl-acyl carrier protein (ACP) thioesterase. However, few of these enzymes can generate a precise product distribution (greater than 90% of a desired chain-length), complicating downstream purification with undesirable blends of alternative chain-length fatty acids. We evaluated several strategies to improve the selectivity of the dodecanoyl-ACP thioesterase from the California bay laurel to produce medium-chain FFAs almost exclusively.

Approach

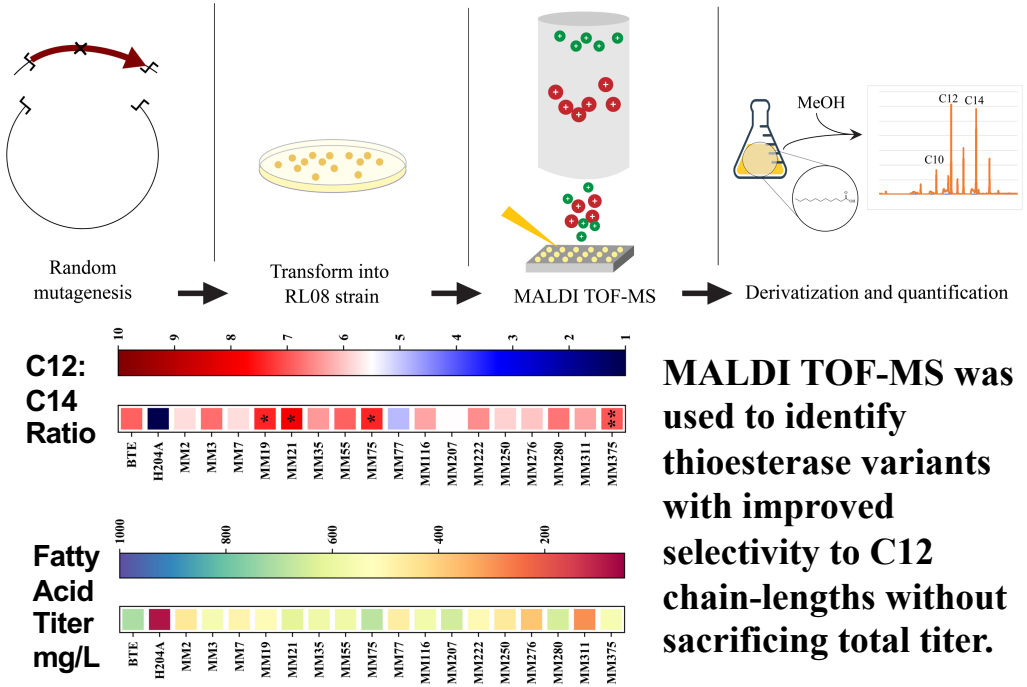
Applied a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) screening technique to identify four thioesterase variants that exhibited a more selective FFA distribution over wildtype in a fatty acid accumulating *E. coli* strain. Then, engineered an *E. coli* strain with these thioesterase variant mutations to increase C^{12} free fatty acid product selectivity.

Results

- Produced free fatty acid C^{12} products with 90% selectivity and 1.9 g/L yield.
- Demonstrated that MALDI-ToF is an effective library screening technique for identifying thioesterase variants with favorable shifts in chain-length specificity.

Significance/Impacts

Demonstrated a strategy of combining synthetic biology and protein engineering to produce medium-chain FFAs, which can translate to oleaginous plants and yeast engineering.



MALDI TOF-MS was used to identify thioesterase variants with improved selectivity to C12 chain-lengths without sacrificing total titer.

Jindra, M.A., Choe, K., Chowdhury, R., Kong, R., Ghaffari, S., Sweedler, J.V., Pfleger, B.F. March 1, 2023. "Evaluation of Strategies to Narrow the Product Chain-Length Distribution of Microbially Synthesized Free Fatty Acids." *Metabolic Engineering*. DOI: 10.1016/j.ymben.2023.02.012.