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MALDI-MS Screening of Microbial Colonies with Isomer Resolution to Select Fatty Acid Desaturase Variants

Background/Objective

Controlling double bond locations in oleochemicals can be a key to producing many value-added products in microbes. However, measuring double-bond locations for improving production is not currently amenable to high-throughput assays due to isobaric masses of the double-bond isomers. We developed a mass spectrometry (MS) assay to determine the positions of double bonds on membrane lipids.

Approach

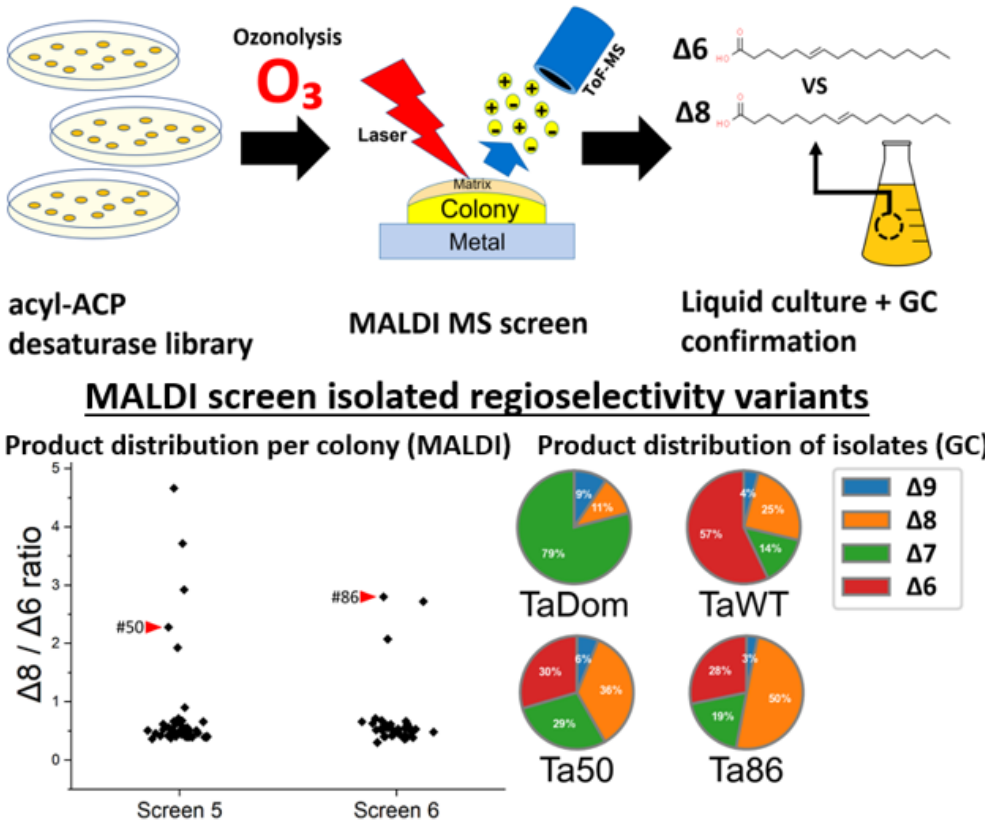
- Agar colonies of *E.coli* expressing a randomly mutagenized library of a C16:1 Δ6 *Thunbergia alata* desaturase gene were treated with ozone.
- Matrix-assisted laser desorption ionization (MALDI) MS was used to measure ozonolysis products of unsaturated fatty acids in each colony for 5 seconds.
- Colonies showing increased C16:1 Δ8, indicating an increase in activity, were validated by liquid culture and gas chromatography (GC).

Results

GC analysis for the liquid cultures of the isolated colonies showed dramatically increased ratio of Δ8 double bonds in the C16:1 species, validating the screen.

Significance/Impacts

This provides a simple and economical high-throughput screening method for improved enzyme activity and metabolic circuits toward producing desired unsaturated fatty acids.



Overall workflow of MALDI-MS screening of the Δ6 desaturase random library and validation of Δ8-active variants by GC-MS.

Choe et al. 2023. "MALDI-MS Screening of Microbial Colonies with Isomer Resolution to Select Fatty Acid Desaturase Variants." *Analytical Biochemistry*. DOI: 10.1016/j.ab.2023.115169.