Lipid Characterization in Genetically Engineered *Yarrowia lipolytica* by Desorption Electrospray Ionization Mass Spectrometry

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OVERVIEW

- The lipid profiles of genetically engineered *Yarrowia lipolytica* strains were analyzed using desorption electrospray ionization mass spectrometry (DESI-MS).
- Characterization of different *Yarrowia lipolytica* strains may allow for the design of a refined and sustainable oleochemical platform targeting the production of specific lipids with industrial relevance.

INTRODUCTION

- Microbial biosynthesis of oils and lipids represents a sustainable source for industrial chemicals precursors. \(^1\) Petroleum sources are unsustainable and raise ethical concerns.
- Engineered *Yarrowia lipolytica* cells can contain greater than 90% lipid composition and titers up to 40 g L\(^{-1}\) lipids. \(^2\)
- The lipid changes resulting from the altered metabolism in the engineered *Yarrowia lipolytica* cells are not fully characterized, although changes in fatty acid composition are known to occur.
- Characterization of lipid changes that occur in combination with increased lipid titers can allow targeted development of a sustainable platform.
- Here, we used DESI-MS to investigate the lipid profiles of two genetically engineered *Yarrowia lipolytica* yeast strains compared to the base strain to characterize the lipid composition and better understand the altered metabolism.

METHODS

**Yarrowia lipolytica** samples
- Three *Yarrowia lipolytica* strains were cultured in triplicate and centrifuged down into cell pellets.
- Samples were deposited in duplicate onto a PTFE printed glass slide and allowed to dry for 30 minutes.

**DESI-MS Analysis**
- A LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, CA) coupled to a commercial DESI-MS platform (Prosciota Inc., IN) was used. In the negative ion mode, DESI-MS was performed with dimethylformamide and acetone (ACN) as the solvent system; in the positive mode, pure ACN was the solvent system. Tandem MS and high mass accuracy measurements were used to identify lipid molecular ions.

**Statistical analysis**
- Spectra from each well were averaged and normalized by total ion current (TIC).
- Principle Component Analysis (PCA) and Significant Analysis of Metarays (SAM) were used to analyze the data and identify molecular ions of interest in the engineered *Yarrowia lipolytica* strains.
- PCA analysis of DESI-MS data allowed for identification of ions that describe the most variance.
- SAM analysis of DESI-MS data allowed directed comparison of ions of statistical interest and upregulation or downregulation in each strain.

**SAM Analysis**
- SAM analysis reveals hydroxy fatty acids that are upregulated in E26 and L36 and po1f.

RESULTS

**Example of MS/MS of Hydroxy Fatty Acids**
- **Positive DESI-MS**
  - Spectra of *Yarrowia lipolytica* in positive ion mode
  - Positive ion mode shows clear differences in the m/z 850-850 region likely consisting of triacylglycerols (TAGs).

**Negative DESI-MS**
- **Spectra of *Yarrowia lipolytica* in negative ion mode**
  - Positive DESI-MS
  - PCA Plot
  - PCA shows clear separation between base strain (po1f) from engineered strains (E26 and L36).
  - PCA analysis suggests batch variation between triplicates of base strain po1f is substantial.

**REFERENCES**


CONCLUDING REMARKS

- DESI-MS allows characterization of lipid changes between genetically engineered strains of *Yarrowia lipolytica*.
- Differences in altered metabolism can be revealed even between strains with similar lipid titers.
- Positive ion mode data analysis will be carried out to characterize lipid species in each strain. Positive and negative ion mode analyses of cell supernatant will also be pursued.
- Ultimately, we expect this information to aid in the development of a refined and sustainable oleochemical platform.

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