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

John Q. Lin[a], Lauren T. Cordova[b], Hal S. Alper[b], and Livia S. Eberlin[a]

[a]*Department of Chemistry, The University of Texas at Austin, Austin, Texas* [b]*McKetta Department of Chemical Engineering, The University of Texas at Austin, Austin, Texas*

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 chemical precursors.[1] Petroleum sources are unsustainable and raise ethical concerns.
- Engineered *Yarrowia lipolytica* cells can contain greater than 90% lipid composition and titer up to 40 g/L 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.

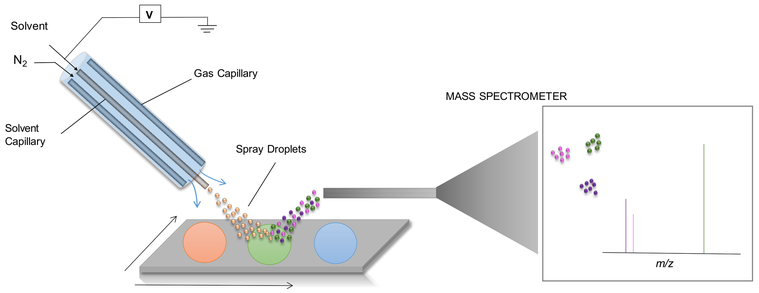
METHODS

Yarrowia lipolytica samples

- Three *Yarrowia lipolytica* strains were cultured in triplicate and centrifuged down into cell pellets.
- Samples were deposited in duplicated 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 (Prosolia Inc., IN) was used. In the negative ion mode, DESI-MS was performed with dimethylformamide and acetonitrile (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.



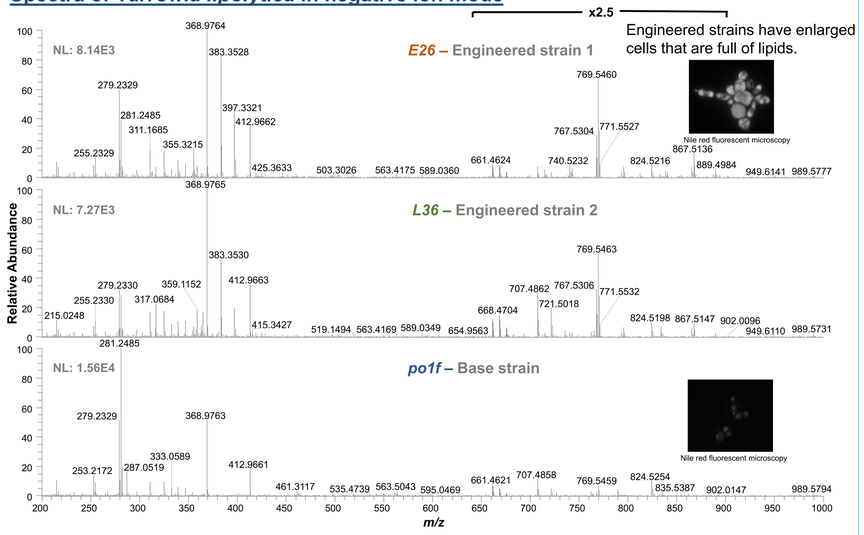
Statistical analysis

- Spectra from each well was averaged and normalized by total ion current (TIC).
- Principle Component Analysis (PCA) and Significant Analysis of Microarrays (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.

RESULTS

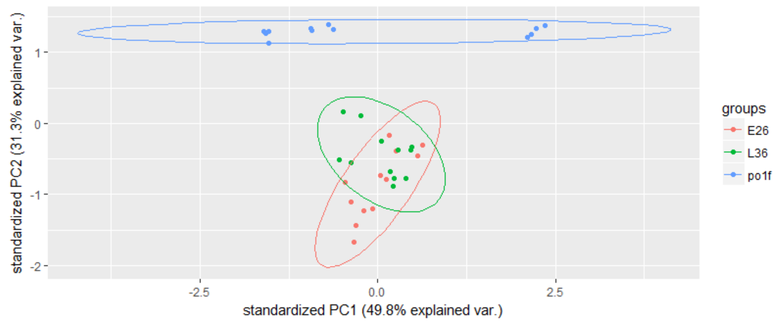
Negative DESI-MS

Spectra of *Yarrowia lipolytica* in negative ion mode



- Engineered *Yarrowia lipolytica* shows accumulation of fatty acids and complex lipids in the *m/z* 700-800 range.

PCA Plot



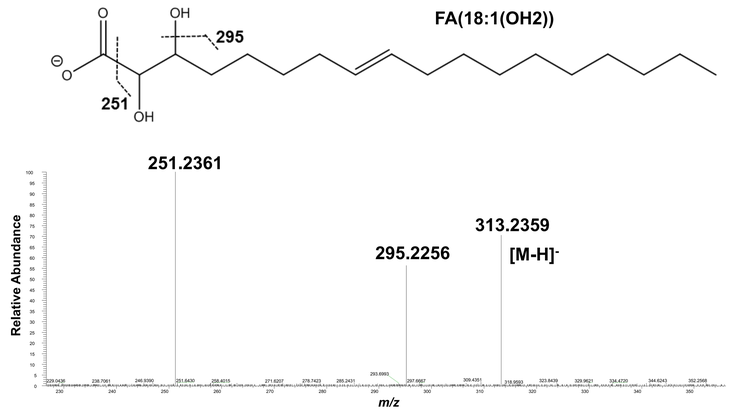
- PCA shows clear separation between base strain (po1f) from engineered strains (E26 and L36).
- PCA analysis suggests batch variation between triplicates of base strain po1f is substantial.

SAM Analysis

Attribution	Formula	Ion Detected *m/z*	Mass Error (ppm)	Contrast Score E26	L36	po1f
FA(17:1(OH2))	C17H31O4	299.2226	0.67	11.133	-5.566	-5.566
FA(18:2(OH2))	C18H31O4	311.2226	0.64	9.335	-4.623	-4.712
FA(16:1(OH2))	C16H29O4	285.2070	0.35	8.324	-4.162	-4.162
FA(18:1(OH2))	C18H33O4	313.2381	0.96	7.383	-3.665	-3.718
FA(20:1(OH2))	C20H37O4	341.2694	0.88	4.463	-2.025	-2.438
FA(24:0(OH))	C24H47O3	383.3528	0.52	3.395	0.718	-4.113
FA(24:0)	C24H47O2	367.3579	0.54	1.381	2.778	-4.159
FA(24:1(OH2))	C24H45O4	397.3320	0.76	3.558	0.014	-3.572
FA(22:0(OH))	C22H43O3	355.3215	0.56	3.734	-0.466	-3.269
FA(20:1)	C20H37O2	309.2796	0.97	3.329	0.183	-3.511
FA(22:1(OH2))	C22H41O4	369.3008	0.54	3.835	-1.335	-2.5
FA(16:2(OH2))	C16H27O4	283.1912	1.06	4.389	-2.195	-2.195
FA(22:2(OH2))	C22H39O4	367.2851	0.82	3.735	-1.864	-1.871
PA(25:0)	C28H54O8P	549.3565	0.55	-2.287	-1.913	4.201
PI(54:9)	C63H104O13P	1099.7236	1.45	-2.22	-1.268	3.488

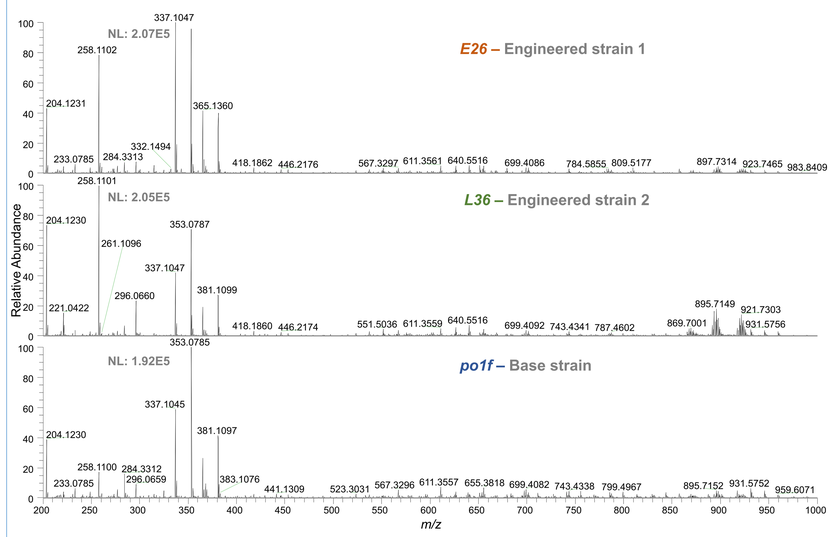
- SAM analysis reveals hydroxy fatty acids that are upregulated in E26 compared to L36 and po1f.

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* 850-950 region likely consisting of triacylglycerols (TAGs).

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.

REFERENCES

1. Curran, K.A. and H.S. Alper, Metab Eng, 2012. **14**(4): p. 289-97.
2. Blazeck, J., A. Hill, L. Liu, R. Knight, J. Miller, A. Pan, P. Otoupal, and H.S. Alper Nat Commun, 2014. **5**: p. 3131.

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