Surviving Starvation: Lipidomic Analysis of Algae Under Nutrient Deprivation

Mary K. Doherty[1], Seshu R. Tammireddy[1], Sarah F. Martin[2], Eliane Salvo-Chirnside[2], Thierry Le Bihan[2], Phillip D. Whitfield[1]

[1]Lipidomics Research Facility, University of the Highlands and Islands, Inverness, United Kingdom, [2]Kinetic Parameter Facility, SynthSys - Synthetic and Systems Biology, The University of Edinburgh, United Kingdom

INTRODUCTION

The unicellular green picoalga *Ostreococcus tauri* is the most primitive known free-living eukaryote.

O. tauri holds a key position at the base of the green lineage of plants, while its very small genome and simple organelle structure make it a very interesting model phytoplankton.

In this study global lipidomic strategies were used in order to define the lipid response of *O. tauri* to nitrogen and phosphorous deprivation.

METHODS: CULTURE CONDITIONS

Wildtype *O. tauri* initially cultured in artificial sea water supplemented with ammonia [NH_4Cl], nitrate [$NaNO_3$], phosphate [β-glycerophosphate], silica, selenium, Keller metals, vitamins and antibiotics for 7 days in 12/12 blue light/dark cycles at 20ºC.

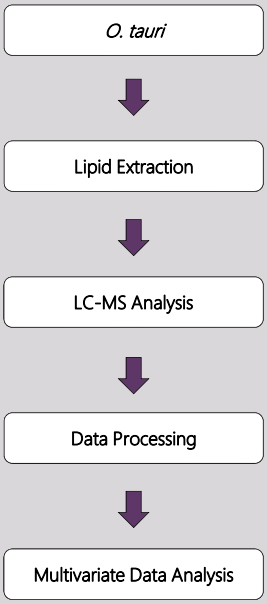
Algae subsequently grown in complete media (P+N+), media lacking nitrogen sources (P+N-) or media lacking phosphorus (P-N+) for 48 hours prior to harvest.

Complete Media
P+N+

Nitrogen Limited
P+N-

Phosphorous Limited
P-N+

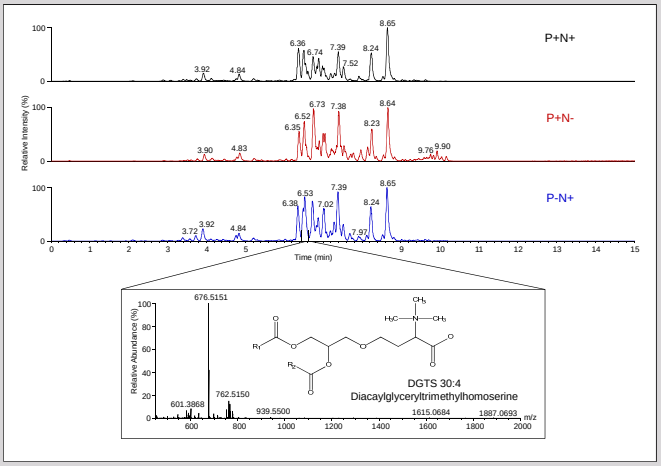
METHODS: ANALYTICAL WORKFLOW



- Lipids from algal samples (n = 3) extracted in chloroform and methanol
- Analysis on Thermo Exactive Orbitrap MS coupled to a Thermo Accela 1250 UHPLC
- Hypersil GOLD C18 column (1.9µm, 100 x 2.1mm) using a ACN/H_2O/IPA gradient over 20 minutes
- Triplicate injections (5µL) in positive and negative ion modes over m/z 200-2000
- Data sets deconvoluted, aligned and normalised using Progenesis CoMet software (Nonlinear Dynamics)
- Principal component analysis (PCA) performed using SIMCA 13 software (Umetrics)

Experimental workflow for comparison of algal lipid profiles

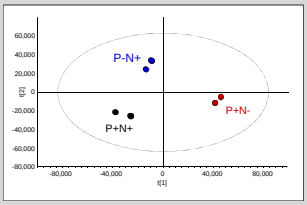
RESULTS: LIPID PROFILING OF ALGAL LIPIDS



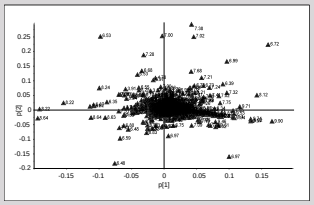
LC-MS analysis (positive ion mode) of lipids from *O. tauri*

RESULTS: DEFINING LIPID CHANGES

PCA Scores Plot



PCA Loadings Plot



Complete Media v P+N-

RT (min)	m/z	Ion	Mass Accuracy (ppm)	Elemental Composition	Lipid ID	Relative Change
9.64	816.7080	$[M+NH_4]^+$	-0.12	$C_{51}H_{90}O_6NH_4$	TAG 48:4	↑ in P+N-
9.71	918.7550	$[M+NH_4]^+$	-0.11	$C_{59}H_{96}O_6NH_4$	TAG 56:9	↑ in P+N-
9.84	844.7391	$[M+NH_4]^+$	-0.36	$C_{53}H_{94}O_6NH_4$	TAG 50:4	↑ in P+N-
9.90	820.7401	$[M+NH_4]^+$	0.85	$C_{51}H_{94}O_6NH_4$	TAG 48:2	↑ in P+N-

Complete Media v P-N+

RT (min)	m/z	Ion	Mass Accuracy (ppm)	Elemental Composition	Lipid ID	Relative Change
6.53	676.5151	$[M+H]^+$	-0.15	$C_{40}H_{70}O_7N$	DGTS 30:4	↑ in P-N+
7.02	704.5462	$[M+H]^+$	-0.43	$C_{42}H_{74}O_7N$	DGTS 32:4	↑ in P-N+
7.24	806.5933	$[M+H]^+$	-0.25	$C_{50}H_{80}O_7N$	DGTS 40:9	↑ in P-N+
7.38	856.6090	$[M+H]^+$	-0.12	$C_{54}H_{82}O_7N$	DGTS 44:12	↑ in P-N+

CONCLUSIONS

Algae grown in nitrogen limiting conditions lead to the production of triacylglycerols whilst betaine lipids are elevated when the algae are deprived of phosphorous.

Understanding the mechanisms underpinning the phenotypic responses at the lipid level may provide further insights into the molecular basis of adaptive plasticity in algae.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of the following: