MS-Based Metabolomics at the Center for Systems Biology

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Photosynthetic Micro-Algae
- Metabolism of pigments involved in photosynthesis
- Hydrophobic Compounds

Systems Biology of Blood Products
- Extension of expiration date of blood products
- Metabolism of stored blood cells
- Small polar metabolites
UPLC-UV-MS$^E$ analysis for quantification and identification of major carotenoid and chlorophyll species in algae.

Photosynthetic Micro-Algae
- Bio-factories producing pharmaceuticals, food additives and cosmetics.
- Metabolism of pigments involved in photosynthesis
UPLC-UV-MS\textsuperscript{E} analysis for quantification and identification of major carotenoid and chlorophyll species in algae.

• Carotenoids and chlorophylls are the major pigment species in higher plants and algae as they have extremely important functions in the photosynthetic pathway being involved in photo protection, light harvesting and reaction center (e.g. photosystem II).

• Understand the effect of light conditions on carotenoid metabolism in algae.

• Identify and quantify these pigments in their respective molecular classes.
Carotenoids

Xanthophylls

Violaxanthin

Antheraxanthin

Lutein

Carotenes

Lycopene

δ-carotene

β-carotene
Chlorophylls

Chlorophyll A

Chlorophyll B
UPLC-UV-MS\textsuperscript{E} analysis for quantification and identification of major carotenoid and chlorophyll species in algae.

ESI-Q-TOF-MS (Synapt G2)
UV detection (450 nm)
UPLC separation (HSS T3 column)

- Separation of Isomers
- Quantification of Targeted Compounds (UV detection)
- MS\textsuperscript{E} - The use of parallel alternating low energy and high energy collision spectral acquisition modes is an alternative approach to data-dependent MS/MS mode.
- Identification of unexpected pigments (Exact mass measurements and fragment ions information)
Acquity UPLC HSS T3 column
1.8 µm (2.1 x 150 mm)
Column temperature: 45°C
elution flow rate: 0.5 mL/min

A: ACN/MeOH/MTBE (70/20/10, v/v/v)
B: 10mM ammonium acetate
60% A - 0 min
75% A - 4 min
100% A - 12 min
98% A - 15 min
60% A - 16 min

1. Neoxanthin
2. Neoxanthin isomer
3. Violaxanthin
4. Antheraxanthin
5. Violaxanthin isomer
6. Zeaxanthin
7. Lutein
8. Lutein isomer
9. Lutein isomer
10. Lutein isomer
11. Chlorophyll b derivative
12. Chlorophyll b
13. Chlorophyll a derivative
14. Chlorophyll a
15. Chlorophyll a’
16. Lycopene
17. δ-carotene
18. α-carotene
19. cis-β-carotene
20. trans-β-carotene
High Energy

Low Energy

Low Energy

UV 450 nm

Lutein

1: TOF MS ES+ 568.4266 0.05Da 9.62e4

1: TOF MS ES+ 1.32e5

Lutein
RT: 9.13

M+ 551.4268

[M+H+H2O]+ 568.4260

145

551.4258

[M+H]+ 569.4327

413.2717

358.2932

439.3210

476.3680

119.0967

175.1486

197.1296

2: TOF MS ES+ 6.61e3

2: TOF MS ES+ 1.54e3

110.07-11.30

9.35, 10.03

9.01

9.13

9.34

10.02

10.03

11.19

9.12
Chlorophyll A

UV
450 nm

Chlorophyll a derivative
RT: 12.70

Chlorophyll a
RT: 13.20

High Energy

4: Diode Array
Range: 3.105e-1
Chlorophyll A Derivatives

- **a**: Chlorophyll a derivative, RT = 11.9
- **b**: Chlorophyll a derivative, RT = 12.3
- **c**: Chlorophyll a derivative, RT = 12.8
- **d**: Chlorophyll a', RT = 13.7
- **e**: Chlorophyll a, RT = 13.3
Chlorophyll B

RT: 11.77

High Energy

UV 450 nm

Chlorophyll b derivative
RT: 11.77

Chlorophyll b
RT: 12.33

597.2011

569.2036

[5+H]^+
907.5211

[5+Na]^+
929.5032

597.1985

569.2045

629.2240

629.2261

629.2240

905.5068

927.4850

929.5060
Chlorophyll B Derivatives

Chlorophyll b derivative
RT = 10.9 min

Chlorophyll b derivative
RT = 11.4 min

Chlorophyll b derivative
RT = 11.9 min

Chlorophyll b'
RT = 12.8 min

Chlorophyll b
RT = 12.4 min
## Quantitative Analysis of 7 Targeted Pigments

### Table 1. Calibration curve, linear range and limit of detection

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope ± SD (n=4)</th>
<th>Intercept ± SD (n=4)</th>
<th>R² ± SD (n=4)</th>
<th>Linear Range (µg/mL)</th>
<th>RSD% lowest point (n=12)</th>
<th>Lod ± SD (ng/mL) (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-β-carotene</td>
<td>2015.5 ± 90.2</td>
<td>135.2 ± 4.1</td>
<td>0.9995 ± 0.0004</td>
<td>0.06-30</td>
<td>9.3</td>
<td>0.033 ± 0.004</td>
</tr>
<tr>
<td>α-carotene</td>
<td>5961.2 ± 179.1</td>
<td>37.1 ± 6.2</td>
<td>0.9993 ± 0.0005</td>
<td>0.0078-4</td>
<td>6.3</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>Lycopene</td>
<td>3496.9 ± 124.5</td>
<td>117.7 ± 3.9</td>
<td>0.9971 ± 0.0016</td>
<td>0.0078-4</td>
<td>10.5</td>
<td>0.031 ± 0.005</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>4026.0 ± 172.0</td>
<td>222.7 ± 27.71</td>
<td>0.9915 ± 0.0008</td>
<td>0.0078-4</td>
<td>8.7</td>
<td>0.021 ± 0.004</td>
</tr>
<tr>
<td>Lutein</td>
<td>5095.2 ± 211.3</td>
<td>326.8 ± 38.4</td>
<td>0.9990 ± 0.0006</td>
<td>0.4-20</td>
<td>5.4</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>2945.6 ± 81.5</td>
<td>683.4 ± 53.3</td>
<td>0.9978 ± 0.0021</td>
<td>0.078-40</td>
<td>6.7</td>
<td>0.018 ± 0.002</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>157.39 ± 12.3</td>
<td>44.1 ± 11.3</td>
<td>0.9992 ± 0.0007</td>
<td>0.2-80</td>
<td>11.8</td>
<td>0.24 ± 0.001</td>
</tr>
</tbody>
</table>
Chlorophyll b and major carotenoids content.

\[ \text{Chlorophyll b} \]

D. salina and carotenoid accumulation under red LED lighting
Proposed pathway of carotenoid metabolism in *D. salina*

- Lycopene
  - Lycopene \(\beta\)- and \(\varepsilon\)-cyclase
  - Lycopene \(\beta\)-cyclase
- \(\alpha\)-Carotene
- \(\beta\)-Carotene
- Lutein
- Zeaxanthin
- Antheraxanthin
- Viola xanthin de-epoxidase
- Viola xanthin epoxidase
- Neoxanthin synthase
- Neoxanthin

Proposed link between carotenoid and chlorophyll metabolism in *D. salina*

- Glycolysis
  - Glyceraldehyde-3-P
  - Isopentenyl-PP
  - Geranyl-geranyl-PP
  - Phytoene
  - Lycopene
  - Carotenoid metabolism
- Glutamate
  - 5-aminolevulinate
  - Chlorophyllide a
  - Chlorophyll a
  - Chlorophyllide b
  - Chlorophyll b
Summary

Carotenoids and Chlorophylls metabolism during different light conditions.

• Separation of isomers in 20 min.

• Quantification of 7 targeted compounds (UV detection).

• Identification of unexpected pigments.
  (Exact mass measurements, fragment ions information and UV detection).
Understanding alterations of platelets metabolism during in vitro storage by using UPLC-Q-TOF-MS strategy.

Blood Products in Transfusion Medicine

- Modern medicine is greatly dependent on the banking and transfusion of blood products.
  - Cancer therapies.
  - Trauma and burn treatments.
  - Obstetrics, and various surgeries.

- Blood components used in these treatments are derived and processed from volunteer blood donors that have undergone a strict screening process.

- Increasingly stringent regulation will lead to a decline in the number of qualified blood donors.

- It is important to extend the expiration date of blood components.

- RBC concentrates are stored for 35-42 days (at 2-6°C).
- PLT concentrates are stored for 5-7 days (22°C with agitation).

- PLT and RBC develop a condition called storage lesions during their storage in plastic blood containers.
Platelet storage lesion (PSL)

- Platelets are small enucleate cells.
- They participate in homeostasis by forming blood clots and serving as a reservoir of different cytokines and growth factors.
- Platelet storage lesion (PSL) leads to a significant loss of platelet function during 5 days storage.
- Changes in platelet morphology.
- Physiological activation.
- Release of platelet granules.
- Platelets lose ability to aggregate.
- Show reduction of matrix adhesion properties.
UPLC-MS strategy for polar metabolites

• Optimization of an UPLC-MS method for metabolite profiling of platelet samples.

• Seven different extraction methods.
  • MeOH:H₂O (7:3) – pH 2
  • MeOH:H₂O (7:3) – pH 7
  • MeOH:H₂O (7:3) – pH 10
  • CH₃CN
  • Hot MeOH (80°C)
  • MeOH:H₂O (7:3) – two step
  • MeOH:CHCl₃:H₂O (6:3:1)

• Two different LC-MS methods (HILIC vs RPLC).
  • HILIC Acquity UPLC Amide Column, 1.7 μm (2.1 x 150 mm)
    • (CH₃CN, H₂O) Acidic condition ESI+/Basic condition ESI-
  • RPLC Acquity UPLC HSS T3 Column, 1.7 μm (2.1 x 150 mm)
    • (MeOH, Formic Acid)

• The coverage of the platelets' metabolome.
• The recovery/response of the identified metabolites.
• The reproducibility of the analytical method.
• The time of the analysis.
Evaluation of extraction processes and chromatographic strategies

Reproducibility and identification of metabolites

Table 1. Number of metabolites tentatively identified in each experimental condition.

<table>
<thead>
<tr>
<th></th>
<th>All conditions</th>
<th>RPLC all extractions</th>
<th>HILIC all extractions</th>
<th>In common (HILIC and RPLC)</th>
<th>Only with RPLC</th>
<th>Only with HILIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPLC</td>
<td>90</td>
<td>93</td>
<td>68</td>
<td>93</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>107</td>
<td>88</td>
<td>105</td>
<td>108</td>
<td>108</td>
</tr>
</tbody>
</table>

Metabolomics of Stored Platelets

3 bags
Each Bag – 5 blood donors

Sample Collection
Day 0-2-4-6-8-10

UPLC-HILIC-MS

Cells/Medium Separation

UPLC-HILIC-MS

Endo-Metabolome

Exo-Metabolome
What kind of data did we get?

138 Metabolites
121 Intracellular Metabolites
105 Extracellular Metabolites

**Lactate**
- **Intensity** vs **Storage Time**
- **Medium** vs **Cells**

**Glucose**
- **Intensity** vs **Storage Time**
- **Medium** vs **Cells**
Multivariate Statistical Analysis

**Focused Principal Component Analysis (PCA)**

PCA assumes that n-dimensional data can be reduced to linear combination of principal components which best explains the variance of data.

**Focused Partial Least Square Discriminant Analysis (PLS-DA)**

Supervised classification methods designed to enhance the separation between groups by rotating the PCA components to achieve maximum separation.

- 227 variables (Extra- and intracellular metabolites).
- 18 observation (6 time points, 3 biological replicates).
- 6 groups (time points).
PLS-DA Intra- and extracellular measurements

- **Day 0**: Initial state
- **Day 2**: Transition point
- **Day 4**: Transition point
- **Day 6**: Transition point
- **Day 8**: Transition point
- **Day 10**: Transition point

**c[2] vs. c[1]**
- **Day 0**: Baseline
- **Day 2**: Early change
- **Day 4**: Progression
- **Day 6**: Peak change
- **Day 8**: Regression
- **Day 10**: Final state
PLS-DA

Intracellular Measurements

1st latent variable
1. ATP
2. NADH
3. Xanthine
4. CoA
5. Glucose-P
6. Choline

2nd latent variable
1. Sedoheptulose-P
2. Glycerol-P
3. Glucose-P
4. Fructose-P
5. Glutathione Ox
6. Succinate

Extracellular Measurements

1st latent variable
1. Xanthine
2. Glutamine
3. Glucose
4. Lactate
5. Glutathione Ox
6. Hypoxanthine

2nd latent variable
1. Fumarate
2. Phosphocholine
3. Tryptophan
4. Malate
5. Glutathione Ox
6. Glycerophosphocholine
Extracellular Measurements

T-Sol solution (pH 7.2)
1000 mL of H$_2$O
Sodium citrate (2.94g)
Sodium acetate (4.08g)
Sodium chloride (6.75g)

20-30% of plasma coming from the blood donors
Extracellular measurements

Consumption

Monotoning Decreasing UPTAKE

Mixed Decreasing TRANSITION POINT
Extracellular measurements

Secretion

Monotoning Increasing Secretion

Mixed Increasing TRANSITION POINT

Lactate

Glutathione

Fumarate

Xanthine

Hypoxanthine

Aconitic Acid
Glycolysis
Pentose Phosphate Pathway
Purine Catabolism
TCA cycle
Glycolysis

Glucose → ADP → G6P → ATP → F6P → ADP → F-1,6-BisP → ATP → 1,3-BPG → ADP → 3-PG → ATP → PEP → ADP → Pyruvate → ATP → Lactate → TCA cycle

Graphs showing changes in ADP, ATP, Glucose-P, ATP/ADP, Fructose-P, and Hexose-Bis-P over storage time.

Day 0, Day 2, Day 4, Day 6, Day 8, Day 10
Pentose Phosphate Pathway

Glucose $\xrightarrow{\text{NADP}^+} \text{NADPH}$

G6P $\xrightarrow{\text{6P-Gluconolactone}}$ 6P-Gluconate

F6P $\xrightarrow{\text{Erythrose-4P}}$ Xylulose-5P

G3P $\xrightarrow{\text{Sedoheptulose-7P}}$ Ribulose-5P

Ribose-5P

Pyrurate

PRPP

Glutathione Ox

G6P $\xrightarrow{\text{NADP}^+}$ 6P-Gluconolactone $\xrightarrow{\text{NADPH}}$ 6P-Gluconate

Ribulose-5P $\xrightarrow{\text{NADPH}}$ Ribulose-5P

Sedoheptulose-7P $\xrightarrow{\text{Ribose-5P}}$ Sedoheptulose-7P

F6P $\xrightarrow{\text{Erythrose-4P}}$ Xylulose-5P

G3P $\xrightarrow{\text{Sedoheptulose-7P}}$ Ribulose-5P

Ribose-5P

Pyrurate

PRPP

Glutathione Ox
Purine Catabolism

ATP
ADP
AMPS
AMP

IMP
XMP
GMP

Inosine
Xanthosine
Guanosine

Adenosine

Hypoxanthine

Adenine

Xanthine

Urate
Summary

HILIC-ESI-MS

1. Exchange of metabolites between intracellular and extracellular environment.
2. Intracellular metabolite profiling.

- Carry-over plasma provides nutrients to sustain platelets metabolism.
- Platelets use glucose and glutamine as main fuel, and secrete large amounts of lactate.
- Accumulation in the supernatant of xanthine and hypoxanthine.
- Damage of mitochondria after day 2.
- Accumulation of pyruvate and lactate.
- Glycolysis and pentose phosphate pathway slow down.
- Oxidative stress.
- Accumulation of AMP, which increases purine catabolism.
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