Dunaliella sp from hypersaline environments: Molecular, phylogenetic and biochemical analyses

Yanan Xu, Declan Schroeder, *Patricia J Harvey

p.j.Harvey@gre.ac.uk
Roadmap

• Introduction to D-Factory
• *Dunaliella* from hypersaline environments
• Taxonomy - historical perspective
• Bar-code approach – modified phylogenetic tree
• Biochemistry
The D-Factory for β-carotene from *Dunaliella*
Natural β-carotene isomers

- Precursor of vitamin A
- Anti-oxidant
- Retinitis pigmentosa
- Atherosclerosis
- Psoriasis
- Anti-inflammatory
- Anti-diabetic

...And more
Bioprospecting
Halotolerant strains of interest to the D-Factory
Uni-algal and clonal Dunaliella culture collection
Salt pans Israel, South Africa, Namibia, Spain, Italy
**Dunaliella** (marine group)

Massjuk (1973) – physiological & biochemical criteria
(chlorophyll $a$ & $b$, lutein, violaxanthin, neoxanthin, $\alpha$ and $\beta$ carotene)

- **Oligo-euhaline** (2-4% NaCl)
- **Hyperhaline** (6-12% NaCl)
  - (Ginzburg & Borowitzka groups)

**Halotolerant** (0.4 – 4% NaCl, but can tolerate up to 34% NaCl)

**Halophilic**

- **Dunaliella salina** (Teodoresco 1905) up to 90% pigments
- **Dunaliella parva** (Lerche 1937)* up to 20% accumulates carotenes
- **Dunaliella tertiolecta** (Butcher 1959)
- **Dunaliella viridis** (Teodoresco 1906)
- **Dunaliella minuta** (Lerche 1937) does not accumulate carotenes
Wilcox et al (1992)--- 18S rDNA

*Dunaliella salina* (Teodoresco 1905) – 1 intron
*Dunaliella parva* (Lerche 1937) – 2 introns

Olmos et al (2000 & 2009)--- 18S rDNA

*Dunaliella salina* (Teodoresco 1905) –
- 0 (CCAP 19/25),
- 1 (Chile & 19/30)
- 2 diff sizes (CCAP 19/18) introns

*Dunaliella bardawil* (Avron & Ben-Amotz 1980) – 2 introns (2500bp)

*Dunaliella tertiolecta* (Butcher 1959) – 0 (UTEX 999 & CCMP 1320) introns

Note: *salina* & *bardawil* are different
Molecular bar-coding

Gonzalez et al 2001 – ITS 1 - ITS2

ITS2, Assuncao et al 2012
Molecular bar-coding

Suite of molecular markers used for the genotyping of isolated strains

- Nuclear: LSU, SSU, ITS
- Chloroplast: *rbcL* (chloroplast) and
- Mitochondrion: *tufA* (mitochondrion)
- ....and intron sizing
Molecular bar-coding
Biochemical characterisation
Effects of light intensity

- Algem photobioreactors
- White LED lights: Four light intensities -
  - 200, 500, 1000 and 1500 μmol m⁻² s⁻¹.
<table>
<thead>
<tr>
<th>CCAP 19/30</th>
<th>UTEX 2538</th>
<th>DF17</th>
<th>DF40</th>
<th>DF15</th>
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<td><img src="image1.png" alt="Light 600x" /></td>
<td><img src="image2.png" alt="UTEX 2538" /></td>
<td><img src="image3.png" alt="DF17" /></td>
<td><img src="image4.png" alt="DF40" /></td>
<td><img src="image5.png" alt="DF15" /></td>
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</table>

**A**

**B**

**C**

Legend:
- **A**: Light 600x
- **B**: Confocal 630x
- **C**: Cultures
Growth rate

Specific growth rate (day\(^{-1}\))

Light intensity (µmol m\(^{-2}\) s\(^{-1}\))

- DF17
- DF40
- UTEX 2538
- DF15

The graph shows the specific growth rate of different algae species (DF17, DF40, UTEX 2538, DF15) in response to varying light intensities. The specific growth rate is calculated as the slope of the growth curve over time. The light intensity is measured in µmol m\(^{-2}\) s\(^{-1}\), and the graph illustrates how the growth rate increases as light intensity increases up to a certain point, after which it plateaus or decreases due to photoinhibition or other physiological limits.
Photosynthesis

\[ \text{DF40 photoinhibition} \]

\[ \text{O}_2 \text{ evolution (nmol O}_2 \text{ hour}^{-1} \text{ cell}^{-1}) \]

\[ \text{Light intensity (μmol m}^{-2} \text{ s}^{-1}) \]

- DF15
- DF40
- DF17
- UTEX2538
Growth: White light, 1500 µmol m⁻² s⁻¹
HPLC

All trans β-carotene
9-cis β-carotene
Cellular content

**All trans β-carotene**

- **200**: DF15, UTEX 2538
- **500**: DF15, UTEX 2538
- **1000**: DF15, UTEX 2538
- **1500**: DF15, UTEX 2538

**9-cis β-carotene**

- **200**: DF15, UTEX 2538
- **500**: DF15, UTEX 2538
- **1000**: DF15, UTEX 2538
- **1500**: DF15, UTEX 2538

Light intensity (µmol m⁻² s⁻¹)

- DF15
- DF17
- DF40
- UTEX 2538
Productivity

All trans β-carotene

9-cis β-carotene

Light intensity (µmol m⁻² s⁻¹)

Productivity (mg L⁻¹ day⁻¹)

DF15  DF17
DF40  UTEX2538
## Algae Biorefineries for Europe – Towards a Sustainable Economy

### Table: 11 traits

<table>
<thead>
<tr>
<th>Light intensity</th>
<th>Strain</th>
<th>all-trans β</th>
<th>9-cis β</th>
<th>glycerol</th>
<th>lutein</th>
<th>Zeaxanthin</th>
<th>all-trans α</th>
<th>respiration</th>
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Algae Biorefineries for Europe – Towards a Sustainable Economy

17-18 October, Brussels
Correlation charts of all examined variables
• Strains differ
Correlation analysis

Distance

Respiration
Lutein
Photosynthesis
Total chlorophyll
Total carotenoids
All-trans β-carotene
All-trans α-carotene
9-cis β-carotene
Car/Chl
Zeaxanthin
Glycerol
PCA analysis

- DF15 (NBT) ↔ UTEX (bardawil)
- DF17 (NBT) ↔ DF40 (Monzon)
- DF15 (NBT) ↔ UTEX (bardawil)
- DF17 (NBT) ↔ DF40 (Monzon)

- Different outcomes
- Both clusterings meaningful

Both tools are useful depending on purpose: strain ownership or production.
NBT, Eilat Israel

Coastal seawater – liquid CO₂ – Typical Strains: D. bardawil, DF15, DF17

MONZON BIOTECH, Spain

Inland salt mine – fluegas CO₂ – Typical Strain: DF40
Strains available from the MBA Culture Collection

The MBA Collection consists of some 400 strains from 80 genera of marine phytoplankton, many of which are not held by any other collection in the world. Within the collection we have a large number of *Emiliania huxleyi* strains and *Dunaliella* species contributed by major international projects.

The Collection plays an essential role in many research programmes funded by the NERC and EC. Research fellows and their collaborators can utilise the collection for their research.
This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613870

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