Genetic modification of microalgae for the sustainable production of high value products

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Metabolic Engineering of Microalgae for Sustainable Production of omega-3 LC-PUFAs

The problem
• There is a growing demand for fishmeal and fish oil
• Alternative lipid and protein sources are required

Potential solution
• Microalgae as a replacement for fishmeal and fish oils in aquafeeds
• Sustainable solution to omega-3 LC-PUFAs and protein supply

Challenges
• To find an algal strain with a high lipid content and a fast growth rate that is easy to harvest;
• A cost-effective cultivation system

Engineering of lipid pathways  Growth optimization  Harvest  A novel aquaculture feed
A unicellular diatom
Accumulates 20 to 30% TAG
Synthesises up to 30% EPA and traces of DHA

- Completed genome sequence
- Established transformation system
- Easy to cultivate
Targeting specific steps of lipid metabolism in *P. tricornutum*

Schematic representation of fatty acid metabolism in *P. tricornutum*

1, 2, and 4: developing strategies to channel EPA into TAGs;
3: Generation of high levels of DHA;
5: Targeting DHA into TAGs
Generation of *P. tricornutum* strain with elevated levels of omega-3 DHA

**pPtOS2**

- **MCS3**
  - fcpAP
  - OtElo5
  - fcpAT
  - fcpAP
  - OtD6
  - fcpAT
  - ble

**Graph:**
- **EPA**
- **DPA**
- **DHA**
- **WT**
- **Elo5**
- **Otd6PtElo5**

**8-fold increase in DHA in transagenic strain**

**Pie charts:**
- **FISH OIL**
  - C16:0
  - C16:1
  - C18:1
  - EPA
  - DHA
  - others

- **PT_ELO5**
  - C16:0
  - C16:1
  - C18:1
  - EPA
  - DHA
  - others
Profiling of TAG molecular species produced in WT and transgenic Elo5 algae using ESI-MS/MS

Cells were grown at 20°C and sampled during stationary phases

In transgenic strain DHA was efficiently incorporated into TAGs
From 50 mL to >1500 L: A scale-up story

- 50 mL flasks
- 10L bubble columns
- 500L PBR “Buttercup”
- 1500L Raceway Pond
- Plymouth Marine Laboratory

PML Plymouth Marine Laboratory

ROTHAMSTED RESEARCH

BBSRC bioscience for the future
Transgenic algae: from small – to large scale

Transgenic strain Pt_Elo5 was successfully cultivated at large scale, demonstrating the efficacy of producing ω-3 LC-PUFAs in Bioreactors and raceway ponds

Hamilton et al., 2016 PloS,
Heterotrophic Production of Omega-3 LC-PUFAs

Photoautotrophic systems

Open pond system  Photobioreactor

The requirement for light to increase cell densities and production is the main objective and a limiting factor of the cultivation

Heterotrophic

Light limitation can be overcome by the cultivation of microalgae under heterotrophic conditions, meaning supplementation of growth media with organic carbon to meet cellular energy requirements

Fermenter
Cells were grown, at 20°C, 100 µmol photons/m²/s

Trophic Conversion of P. tricornutum by transformation with a gene encoding a glucose transporter

Alternative technologies for the production of ω-3 LC-PUFAs have been developed

Hamilton et al., 2016, Marine Drugs
We have engineered the diatom *Phaeodactylum tricornutum* to accumulate elevated levels of the high value omega-3 LC-PUFA DHA in photo- and heterotrophically grown strains.

DHA was shown to accumulate in TAG, with levels remaining stable at different growth stages up to 1,500 l culture volumes.

These studies show the opportunity to establish *P. tricornutum* as a synthetic biology chassis for enhancing LC-PUFAs production in microalgae.
Future directions

- Using the RRes lipidomic platform we will continue our study of the biosynthesis of valuable lipids in microalgae to provide further knowledge for manipulating pathways for industrial applications.

- We will screen available biodiversity in order to identify novel algal strains for the production of ingredients and proteins for food and feed, and of chemicals for pharmaceutical, cosmetics and nutraceutical applications.
Algal oils: PALM-UK

Mary Hamilton        Chloe Economou
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Richard Haslam
Johnathan Napier

Thank you!