Increased lipid accumulation without compromising growth: Metabolic engineering of lipid catabolism in *Thalassiosira pseudonana*
Engineering microalgae for fuel production

Metabolic engineering

Production of non-GMO strains

SD-CAB
Biofuels from algae

Many microalgae accumulate lipids that can be converted to fuel

Microalgal cell → Lipids accumulating → Biofuel

Nils Kroger

Hildebrand Lab

1800recycling.com
Lipid accumulation in microalgae

Lipid yields are a product of both lipid accumulation and biomass

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<th>Lipid/cell</th>
<th>Number of cells</th>
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Lipid metabolism

Lipid catabolism

Disrupting lipid catabolism can lead to increased lipids without deleterious effects on growth.
Lipid catabolism

- TAG lipid droplet
- FFAs
- Lipases
- Phospholipases
- Peroxisome
  - β-oxidation
  - Acetyl-CoA + LCFA
  - Glyoxylate Cycle
- Mitochondrion
  - β-oxidation
  - Acetyl-CoA
  - TCA cycle
Transcriptomics-guided target identification

Lipid accumulation in *Tp* under Si starvation

Lipid accumulation in *Tp* under Si starvation
Overview of lipase expression

Fold change in expression

Time after Si limitation (hrs)
Target: Thaps3_264297

Fold change in gene expression

Thaps3_264297 gene expression

WT lipid accumulation

Fold change in BODIPY fluorescence

Hours after Si limitation
Functional characterization of Thaps3_264297

### Phospholipase activity

**Values:**
- AD: 620 units phospholipase activity/mg protein
- Thaps3_264297: 600 units phospholipase activity/mg protein

### Lipase activity

**Values:**
- AD: 8 units lipase activity/mg protein
- Thaps3_264297: 8.5 units lipase activity/mg protein

### Acyltransferase activity

**Reaction:**
- LPA + oleoyl-CoA → PA

**Stained gel:**
- Solvent front
- PA
- Origin

![Image of acyltransferase activity gel](image-url)
Knock-down of Thaps3_264297

**Immunoblotting**

**Functional assay**

- *p<0.05
- **p<0.01
- ***p<0.001
Growth analysis

Antisense strains 1A6 and 1B1 show no decrease in growth

* p<0.05
Growth analysis

Antisense strains 1A6 and 1B1 show no decrease in growth
Lipid analysis

1A6 and 1B1 show increased TAG in stationary phase

E: exponential S: stationary
Lipid analysis

1A6 and 1B1 show increased lipid accumulation during nutrient starvation.

*\textit{p}<0.05
Lipid analysis

1A6 and 1B1 show increased lipid accumulation during nutrient starvation
Lipid analysis

1A6 and 1B1 show increased lipid accumulation during nutrient starvation

**Corine Glé***

Aaron Hartmann

***p<0.001

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Cell intactness

Transgenic cells were visibly more intact after nutrient limitation
1A6 and 1B1 show decreased membrane permeability during nutrient starvation.

SYTOX fluorescence

WT
1A6
1B1

SYTOX fluorescence/cell (arbitrary units)

0
1
2
3
4
5
6
7
8
9

8 48

Time after Si starvation (hrs)

*p<0.05
**p<0.01
Membrane stability

1A6 and 1B1 show decreased membrane permeability during nutrient starvation

**Polar lipids**

**SYTOX fluorescence**

* p<0.05
** p<0.01
Membrane stability

1A6 and 1B1 show decreased membrane permeability during nutrient starvation.
Summary

Transcriptomics guided identification of target Thaps3_264297

Thaps3_264297 is homologous to CGI-58

Thaps3_264297 has lipase, phospholipase and acyltransferase activities

Knock-down strains 1A6 and 1B1 show uncompromised growth, increased lipids in exponential and stationary phase, increased lipids during nutrient starvation, and more intact membranes
Engineering lipid metabolism

Algenol
Sapphire Energy

Solix Biosystems
SD-CAB
Engineering lipid metabolism
Selectable markers

Knock-down strains 1A6 and 1B1 show uncompromised growth, increased lipids in exponential and stationary phase, increased lipid yields during nutrient starvation, and more intact membranes.
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