Algal Biofuels Research: Using basic science to maximize fuel output

Jacob Dums, PhD candidate, jtdums@ncsu.edu
Heike Sederoff Lab
March 9, 2015
Outline

- Research Approach
- *Dunaliella*
- Increase Oil Content by Genetic Engineering
- Effects of Light and Temperature
- Using Amino Acids as a Nitrogen Source
- Current Work
- Summary
Basic vs Applied Research

• Basic Research
  – A form of systematic study directed toward greater knowledge or understanding of the fundamental aspects of phenomena
  – Research for science’s sake

• Applied Research
  – a form of systematic inquiry involving the practical application of science
  – Research to solve a specific problem
Consequences of the Environment, Basic Research

- Development
  - Cell Division
  - Sexual Reproduction
  - Cell Size
  - Transcription

- Metabolism
  - Oil storage
  - Sugar storage
  - Protein production
  - Chlorophyll

- Pressure
- Nitrogen
- CO₂
- pH
- Salt
- Radiation
- Phosphate
- Light
- O₂
- Temperature
Maximizing Output, Application Research

- Decreasing Input
  - Growing space
  - Light
  - Time
  - Nutrients

- Increasing Output (Money)
  - More oil
  - Better harvesting
  - Secondary byproducts
Dunaliella

- Small unicellular green alga (~10µm)
- Halophilic, isolated from high salt environments
- Lacks cell wall
- Currently cultivated for carotenoid production
- 1 partially sequenced genome
  - Available transcriptomes


*Dunaliella viridis*
Expression of extremophile thioesterase A (TesA) in *D. viridis*

- Increase oil content by removing inhibition

**Diagram:**
- Light + CO₂ → Photosynthesis → Triose phosphate → Acetyl-CoA → Acyl-ACP thioesterase → Free fatty acid
- ER → TAG → DAG
- CsTesA
- Fatty acyl-ACP → Acyl-ACP thioesterase → Free fatty acid

Effects of Light and Temperature

- Cells grown under normal or continuous light
- Two different temperatures, 25°C or 35°C
- Continuous light increases cell division
- Short term temp increase has no effect on cell division

Fig. 2: Cell division rate and cell size increases under LL versus LD cycles. *D. viridis* cultures were grown either under LD (dashed lines) or LL (solid lines) at 25°C for the first 24 hours and then either remained at 25°C (blue lines) or the temperature was raised to 35°C (red lines). The temperature shift occurred after 24hrs (black arrow). Cell counts were measured in three biological replicates with three technical replicates each. The error bars represent the standard deviation. Statistical significance was assessed by unpaired, two-tailed, Student's t-test. The values with the same letters are not significantly different at 54 hrs (p<0.05).
Effects of Light and Temperature

- Cells grown under normal or continuous light
- Two different temperatures, 25°C or 35°C
- Both light and temperature cause increased oil content

Fig. 5. TAG content increases under LL (A) and saturated FAs accumulate at 35°C in TAG (B). *D. viridis* cultures were grown either under LD (dashed lines) or LL (solid lines). All cultures were grown at 25°C for the first 24 hours and then either remained at 25°C (blue lines) or the temperature in the growth chamber was raised to 35°C (red lines). The temperature shift is indicated by the black arrow. Total TAG content was calculated as the sum of the fatty acids from the TAG fraction. Total TAG content was normalized to 1 million cells. The error bars represent the standard deviation from three independent biological replicates. Statistical significance was assessed by unpaired, two-tailed, Student’s t-test. The values with the same letters are not significantly different at 54 hrs (p<0.05).
Closing the Loop

10L reactor

LED / natural light

CO₂ / air

recycled seawater

Microalgae separation

Osmotic Lysis

Freshwater backwash

lipid / nutrient separation

nutrient recycle digester

recycled nutrients

pump

seawater holding tank

seawater

Freshwater

OIL

nutrient recycle

Digester

Closing the Loop
Oil Contains No Nutrients

Glycerol

A free fatty acid

Glycerol + 3 fatty acids = fat/oil

http://www.scienceinafrica.com/pics/12_2006/oil1.jpg

http://chemed.chem.purdue.edu/genchem/topicreview/bp/ch10/graphics/10_24.gif
**D. viridis** Utilizes Few Amino Acids

Growth supported only by glutamine, tryptophan, cysteine, and histidine.*

*True at concentrations of 5mM.
Glutamate + NH$_4^+$ → H$_2$O

Oxidase

Glutamine

Amino Acid Transporters

GS-GOGAT Pathway
Growth on glutamine increases cell division and shows no significant effect on oil accumulation.
Growth on Glutamine

Decreases in Chlorophyll and Protein Content may be due to carbon heterotrophy and decreased need of photosynthesis and carbon fixation.
Current Work, Applied

• Increasing Utilization of Amino Acids
  – Expressing additional amino acid transporters
  – Expressing the amino acid oxidase from *Chlamydomonas*

Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val
Current Work, Basic

- Metabolic consequences of increased amino acid utilization
- Characterization of amino acid transporters that *D. viridis* already has
Summary

• Applied vs Basic Research
  – Both are important
  – Basic research gives a basis for applied research
  – Applied research translates discoveries into practical applications

• Algae Research
  – Can use high temp and continuous light to induce oil accumulation and increase growth rate
  – Amino acids support growth, but there are unknown metabolic consequences
Questions?