Multi-scale Characterization of Improved Algae Strains

Dr. Taraka Dale
Bioscience Division
Challenges Remain for Creating a Viable Algae Biofuels Industry

1. **Productivity**. Strains must be improved for productivity to be economically viable. Biomass & lipid productivity are the main drivers of cost.

2. **Consistency**. Strains that have shown improved productivity in the lab environment often perform differently when grown outside.
Project Goal and Objectives

The overall goal of this project is to develop a streamlined process for improving algae strains and characterizing their performance at multiple scales, from the bench to outdoors.

1. Generate improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses

2. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions

3. Transition strains to outdoor ponds for testing
Algae Cultures are Characterized Using Multi-Parameter Flow Cytometry Assays

- Cells are focused in a flowing sample stream for individual interrogation
- Light scatter & fluorescence by the cell is detected & utilized for characterization
- Different populations within heterogeneous samples can be identified & sorted

<table>
<thead>
<tr>
<th>Forward Light Scatter</th>
<th>Side Light Scatter</th>
<th>Red Fluorescence</th>
<th>Green Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Granularity</td>
<td>Chlorophyll</td>
<td>Lipids (when stained)</td>
</tr>
</tbody>
</table>
Subpopulations with Improved Lipid Accumulation Have Been Isolated

Fluorescence-Activated Cell Sorting

Counts

Rounds of Sorting

Grow Sorted Populations

Parent

Sorted-H

Increase in Lipid Content, Fold Over Parent

UNCLASSIFIED
We Focused on Four Subpopulations of *Chlorella sorokiniana* 1228

**Rounds of Sorting**

**Flask conditions:**
- 25°C
- 800 µmole/m²-s²
- 16:8 light:dark

**Populations Selected:**
- Parent
- BD2
- BD4
- ABD2
- AR4

**BODIPY Fluorescence (RFU, minus Unstained)**

- #1
- #2
- #3
A Pipeline for Characterizing Improved Strains at Multiple Scales Was Established

Lab-Scale Strain Improvement & Characterization
- LANL: Strain improvement
- PNNL: Extensive characterization at flask level

Productivities Predicted by the Strain Specific BAT
- PNNL: Generate predictions of areal productivities (g/m²-day)
- Generate light/temp scripts for outdoor simulations

Further Improvement of the Model

Outdoor Conditions Tested Indoors at Lab-Scale
- LANL: Use scripts in Phenometrics ePBRs
- PNNL: Use outdoor testbeds to grow LANL strains

Strains Tested Outdoors
- ATP³: Use outdoor testbeds to grow LANL strains

PNNL
- Characterization for biomass growth model input

LANL
- Generate predictions of areal productivities (g/m²-day)
- Generate light/temp scripts for outdoor simulations

PNNL
- Extensive characterization at flask level
- Use scripts in indoor, environmentally controlled ponds to grow LANL strains
- Use scripts in Phenometrics ePBRs

LANL
- Strain improvement
- Extensive characterization at flask level

PNNL
- Characterization for biomass growth model input

ATP³
- Use outdoor testbeds to grow LANL strains

ATP³
- Algae Testbed Public-Private Partnership
Two Cultivation Systems Simulate Outdoor Conditions with Light/Temperature Scripts

LANL/NMC ePBR Matrix Phenometrics™

Environmental Photobioreactors

PNNL Environmentally Regulated Ponds

- Programmable scripts: Light/temp/pH/CO₂
- Full sunlight intensity, light from above

PNNL Biomass Assessment Tool Predicts C. sorokiniana 1412 Productivity for ATP³ Site
Two Cultivation Systems Simulate Outdoor Conditions with Light/Temperature Scripts

LANL/NMC ePBR Matrix Phenometrics™
Environmental Photobioreactors

PNNL Environmentally Regulated Ponds

- Programmable scripts: Light/temp/pH/CO₂
- Full sunlight intensity, light from above

PNNL Biomass Assessment Tool Predicts C. sorokiniana 1412 Productivity for ATP³ Site
Growth of Lipid-Improved *Chlorella* Strains at Simulated ATP$^3$ Site Was Measured Using ePBRs

Simulated the month of May at the ATP$^3$ location (Mesa, AZ), using the LANL indoor ePBRs

- Parent
- AR4
- ABD2
- BD4
- BD2
Growth of Lipid-Improved *Chlorella* Strains at Simulated ATP$^3$ Site Was Measured Using ePBRs

Simulated the month of May at the ATP$^3$ location (Mesa, AZ), using the LANL indoor ePBRs.
Going Outside, A Collaboration with ATP³

- ATP³ is the Algae Testbed Public-Private Partnership, a testbed facility funded by DOE
- Multiple types of outdoor cultivation facilities
- Our main goal currently is to use open ponds similar to those used for the PNNL indoor ponds (800-1000L)


C. sorokiniana Parent and Sorted Were Cultivated at ATP³ During the Month of May

• Outdoor pond cultivation (3x 1000L) of C. sorokiniana parent and sorted BD4 began May 1, to match simulation conditions.

• In-house analyses and samples sent to LANL, for consistency in analytical methods
C. sorokiniana Cultivation in Three Different Systems with Similar Environmental Conditions

- The indoor data brackets the outdoor data, consistent with data from other strains.
C. sorokiniana Cultivation in Three Different Systems with Similar Environmental Conditions

LANL ePBRs
- ePBR Parent: 11 g/m²-d
- ePBR BD4: 12 g/m²-d

Day of Script (Mesa, AZ in May)

PNNL Indoor Ponds
- PNNL Parent
- PNNL BD4: 13 g/m²-d
- 21 g/m²-d

Day of Script (Mesa, AZ in May)

ATP³ Outdoor Ponds
- ATP3 Parent
- ATP3 BD4: 15 g/m²-d
- 14 g/m²-d

Actual Day in May (Mesa, AZ)

- The indoor data brackets the outdoor data, consistent with data from other strains.
- Areal productivity (g/m²-d) is calculated by the rate of AFDW accumulation during linear growth and the pond depth (25cm). Values are similar except PNNL BD4, which is being repeated.
C. sorokiniana Cultivation in Three Different Systems with Similar Environmental Conditions

- The indoor data brackets the outdoor data, consistent with data from other strains.
- Areal productivity (g/m²-d) is calculated by the rate of AFDW accumulation during linear growth and the pond depth (25cm). Values are similar except PNNL BD4, which is being repeated.
- There is a consistent increase in total accumulated biomass across all systems.
Sorted Culture Has At Least Comparable Areal Productivities & Accumulates >20% More Biomass

Areal Productivity

- Parent
- BD4

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Parent</th>
<th>BD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5L Indoors</td>
<td>12.5</td>
<td>13.2</td>
</tr>
<tr>
<td>800L Indoors</td>
<td>18.7</td>
<td>20.4</td>
</tr>
<tr>
<td>1025L Outdoors</td>
<td>15.0</td>
<td>17.2</td>
</tr>
</tbody>
</table>

May Conditions, Mesa, AZ

Total Biomass

Average Final AFDW (mg/L)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Parent</th>
<th>BD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5L Indoors</td>
<td>475</td>
<td>515</td>
</tr>
<tr>
<td>800L Indoors</td>
<td>1125</td>
<td>1355</td>
</tr>
<tr>
<td>1025L Outdoors</td>
<td>1350</td>
<td>1650</td>
</tr>
</tbody>
</table>

May Conditions, Mesa, AZ

Preliminary Lipids

- ePBRs: 18%
- PNNL: 33%
- ATP: 10-40%

- Relative behavior of the populations is maintained.
- BD4 appears to maintain an improved phenotype at larger scales.
Project Goal and Objectives

The overall goal of this project is to develop a streamlined process for improving algae strains and characterizing their performance at multiple scales, from the bench to outdoors.

1. Generate improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses
   - FACS-sorted *C. sorokiniana* populations

2. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions
   - Additional validation of ePBRs, PNNL indoor ponds, BAT against outdoor data

3. Transition strains to outdoor ponds for testing
   - Improved phenotype appears to be maintained across scales and into a true outdoor environment (prelim.)
Acknowledgments

- Dr. S. Twary
- Dr. A. Barry
- C. Sanders
- M. Teshima
- R. Yoshida
- Dr. S. Starkenburg
- Dr. B. Marrone

- Dr. M. Huesemann
- Dr. M. Wigmosta
- Dr. J. McGowen

Annual Operating Plan

Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE