SAND2017-0579C

# Engineering Green Algae for High Biomass Productivity

*Anne M. Ruffing, Jordan T. McEwen, Todd W. Lane, Pamela D. Lane, and Lucas M. Strickland*

## Problem Statement

Algae are excellent candidates for providing a renewable source of liquid fuels with an overall reduction in carbon emissions compared to conventional petroleum fuels. However, the economic viability of algal biofuel production at industrial scales is limited by the low biomass productivities of natural algal species. While algae rely on photosynthesis to fix $CO_2$ into metabolites for biomass generation, several other pathways act in a reverse manner, leading to the loss of biomass and release of $CO_2$.

At night, algae activate the process of dark respiration, which allows the algal cells to continue the process of cell division but also leads to the loss of fixed carbon (Figure 3). In the light, photorespiration also results in the loss of fixed carbon and other cellular resources (Figure 2). Together, these two metabolic processes account for a 35-115% reduction in biomass productivity.

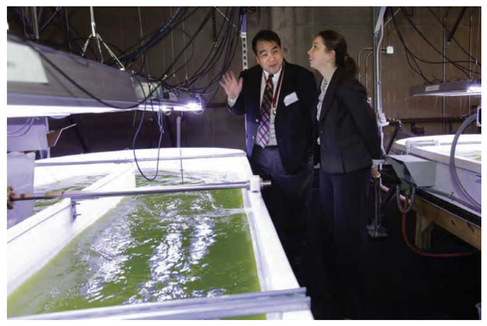

Figure 1. Algal Testbed at SNL, includes three 1,000 L raceway ponds with LED lights and temperature control to simulate outdoor conditions.

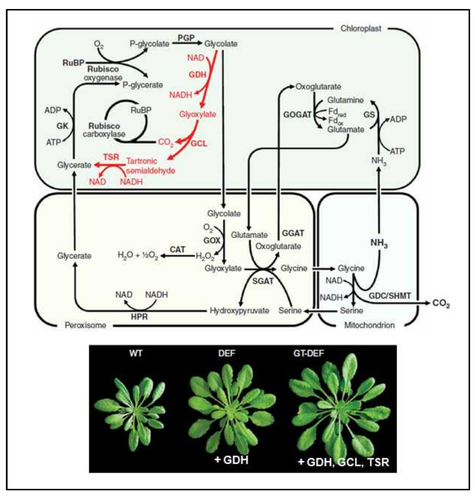


Figure 2. Genetic modification of plants with bacterial photorespiratory bypass pathway to reduce carbon loss associated with photorespiration.[1]

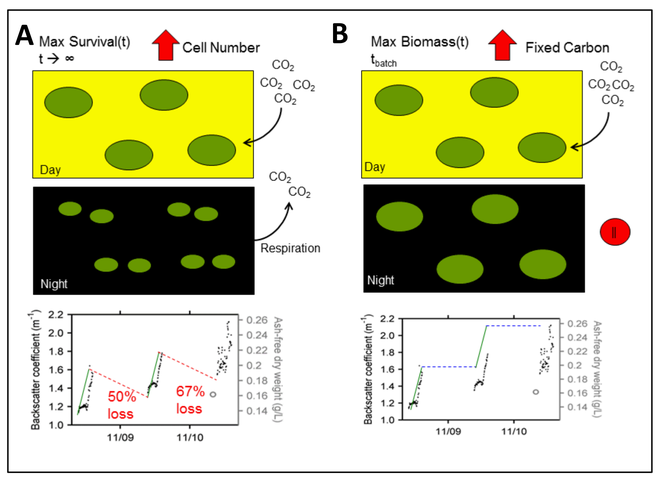


Figure 3. Carbon loss due to dark respiration in *Nannochloropsis salina* (A) and proposed *Nannochloropsis* strains with genetic modification of dark respiration (B).[2]

## Hypothesis and Objectives

We hypothesize that reducing carbon loss from dark respiration and photorespiration through the genetic modification of algae will result in improved algal biomass productivities. We will test this hypothesis through the following objectives:

1. To develop CRISPR/Cas9 genome editing tools for *Nannochloropsis* species (Figure 4)
2. To investigate dark respiration using chemical inhibitors
3. To investigate glycolate production and excretion in *Nannochloropsis* species
4. To develop modified strains of *Nannochloropsis* with reduced dark respiration using CRISPR/Cas9 tools (Table 1)
5. To develop modified strains of *Nannochloropsis* with reduced photorespiration using CRISPR/Cas9 tools (Table 1)
6. To demonstrate scale-up potential of modified *Nannochloropsis* using SNL Algal Testbed (Figure 1)

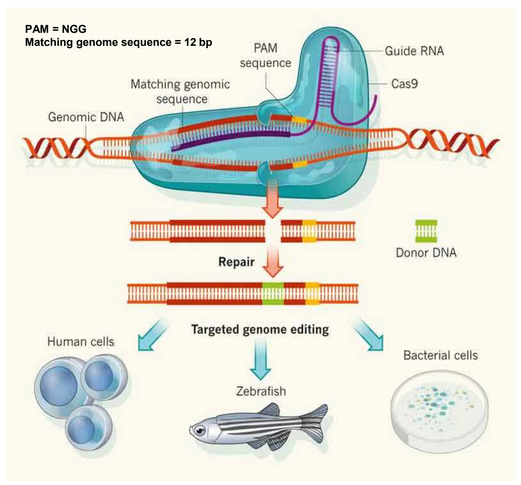


Figure 4. Schematic of CRISPR/Cas9 genome editing in eukaryotes.[3]

Table 1. List of genetic targets in *Nannochloropsis gaditana*

| Pathway | Target Description | Gene Target |
|---|---|---|
| Laminarin degradation | Laminarinase | Nga02655 |
| TAG degradation | TAG lipase CrLIP1 (Chlamydomonas reinhardtii) | Nga01367 |
| | TGL3/TGL4/TGL5 (yeast) SDP1 (Arabidopsis thaliana) | Nga03028 |
| Dark respiration | Cytochrome c oxidase COX1 | Nga50030, Nga50029 |
| | Alternative oxidase AOX1 | Nga03289 |
| Photorespiration | Glycolate dehydrogenase | glcD (E. coli) |
| | Glycolate carboxyligase | glcE (E. coli) |
| | Tartronic semialdehyde reductase | glcF (E. coli) |

## Results: Genetic Tool Development

- Transformation (transfer of DNA across the cell wall) is challenging for algae due their tough algal cell walls.
- High voltage electroporation has been optimized for delivery of DNA into *Nannochloropsis* species (Figure 5).
- However, transformation across different species of *Nannochloropsis* is highly variable (Figure 6).

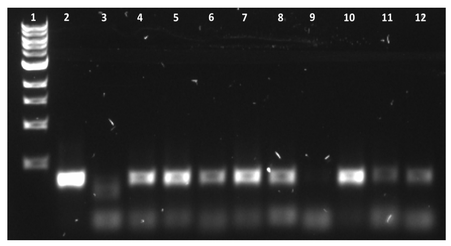


Figure 5. PCR screening of *ble* integrated into the *N. gaditana* genome via high voltage electroporation. Lane 1 = 1kb ladder, Lane 2 = pJTM008 (positive control), Lane 3 = *N. gaditana* gDNA, Lanes 4-12 = zeocin resistant colonies. Expected *ble* fragment = 368 bp.

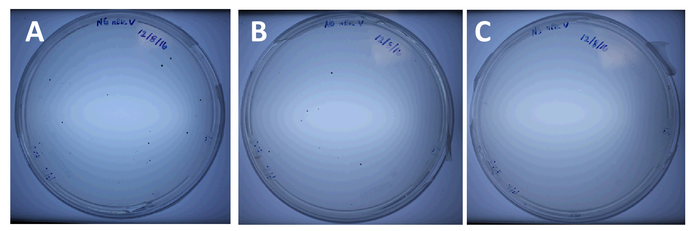


Figure 6. Zeocin resistant colonies of *N. gaditana* (A), *N. oceanica* (B), and *N. salina* (C) after high voltage electroporation of plasmid containing *ble*.

- Introduction of Cas9 leads to reduced transformation efficiencies, suggesting Cas9 expression is toxic in *Nannochloropsis* (Table 2).

Table 2. Transformation of linearized plasmids into *N. gaditana*

| Plasmid Construct | Number of Transformed Colonies |
|---|---|
| pBleT | 19 |
| pBleV | 23 |
| pBleT-Cas9 | 6 |
| pBleV-Cas9 | 9 |

## Results: Physiological Measurements of Dark Respiration and Photorespiration

- Addition of SHAM (salicylhydroxamic acid), inhibitor of dark respiration, resulted in reduced growth (Figure 7).
- SHAM may also inhibit glycolate metabolism, as demonstrated in other algae.[4]

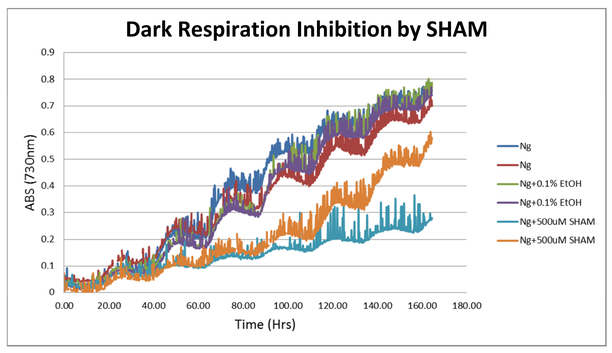


Figure 7. Growth of *N. gaditana* with addition of 500 μM of SHAM. Growth temperature = 25°C; Illumination = 1000 μE; 12h:12h Light/Dark.

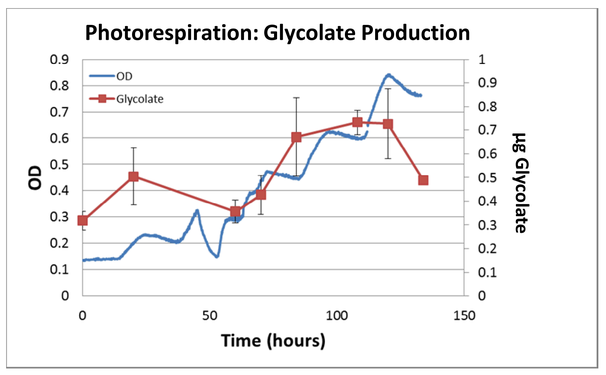


Figure 8. Growth of *N. gaditana* (blue) and glycolate excretion (red). Growth temperature = 25°C; Illumination = 1000 μE; 12h:12h Light/Dark.

- Low levels of glycolate measured in cultures of *N. gaditana* (Figure 8).
- Suggests low levels of photorespiration under the conditions tested.

## Conclusions and Future Work

- Cas9 appears to be toxic to *Nannochloropsis* species.
- Various promoter constructs will be tested to optimize Cas9 expression (Joint Genome Institute DNA Synthesis project).
- Genetic modification of dark respiration genes will determine the effect of dark respiration on carbon loss.
- Photorespiration losses in *N. gaditana* may be low.

## References and Acknowledgements

1. Kebeish et al. (2007) Nature Biotech.25(5):593-599.
2. Reichardt T. ATP3 data for Nannochloropsis salina in mini-raceway ponds at ASU.
3. Charpentier and Doudna. (2013) Nature. 495: 50-1.
4. Goyal A. (2002) Physiologia Plantarum. 116: 264-270

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

*Exceptional service in the national interest*

U.S. DEPARTMENT OF ENERGY

Sandia National Laboratories