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Genetic Tool Development for the green algae, Nannochloropsis

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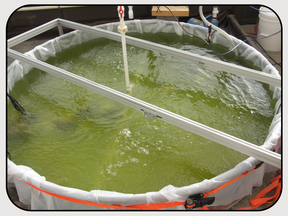
Abstract

Energy and environmental concerns have led to a growing interest in engineering microorganisms for biologically derived fuels and chemicals. A variety of microalgae have been studied as native producers of lipids that can be readily converted to biodiesel. Owing to a lack of well-established genetic tools and techniques, algae have largely been left out of recent advances wherein metabolic pathways are engineered for enhanced fuel and chemical production. Traditional tools for genetic modification have shown to be relatively ineffective for many species of microalgae that are otherwise well-suited for biofuel production. To remedy this condition, we are developing modern recombinant DNA tools for efficient metabolic engineering of a variety robust, marine alga in the genus *Nannochloropsis* . Algae in the *Nannochloropsis* may become an ideal platform organism for tool development since several genomes have been sequenced and rudimentary genetic transformations have been reported.

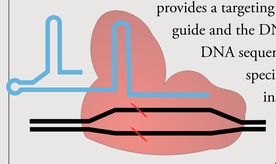
Introduction

Biofuels and chemicals are a promising source of carbon based molecules that may be dropped into the present non-sustainable, petrochemical-based energy and manufacturing system. This may result in steps towards the development of a sustainable and environmentally-friendly economic system. All biobased molecules are made by capturing and storing light energy as carbon-based chemical bonds. Photosynthetic microorganisms are ideal carbon capturing and conversion agents owing to minimal land and nutrient requirements and broad temperature rages. Algae are eukaryotic microorganisms that grow in both fresh and saltwater, with many strains showing the robust growth required for heavy aquaculture use. Algal biomass can be harvested and converted into a fuel, wherein the fuel can be dropped into standard internal combustion system wherein chemical bonds are broken, energy is released and utilized, and carbon dioxide is expelled. Another possible use of algal biomass as a raw carbon resource to make a variety of petrochemical replacements . Microalgae strains have many advantages in carbon capture and recycling; however, the productivity of carbon capture is too low to economically compete with petroleum based fuel and chemicals Modern recombinant DNA techniques and metabolic engineering have been used to endow microorganisms with additional capabilities including modified growth rates and production of non-native metabolites. Applying these tools to micro algae has yet to be fully investigated and developed, leading to a lack of genetic tools and techniques. To remedy this condition, we are developing modern recombinant DNA tools for efficient metabolic engineering of a robust marine algae of the genus *Nannochloropsis.* We are investigating three strains of *Nannochloropsis* in an efforts to build an ideal platform for tool development for algal genomic engineering.



Scale-up Photobioreactor at Sandia National Labs

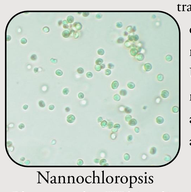
Many eukaryotic algae have proven resistant to traditional manipulation techniques. CRISPR-Cas9 technology is a recently developed tool based on an adaptive immune system of prokaryotes wherein a bacterial endonuclease (Cas9) targets a DNA sequence by way of a programmable guide RNA. The RNA guide strand docks with the Cas9 and provides a targeting template relying on canonical Watson-Crick base pairing between the RNA guide and the DNA target. When the Cas9-RNA complex interacts with the complementary DNA sequence, the endonuclease domain induces a specific double-strand break. This specific break allows for gene knockouts via non-homologous end joining or insertions via homology directed repair. In this work, we attempt to optimize transformation methods, increase rate of random integration of an antibiotic resistance cassette and heterologously express Cas9. Once a stable genetic mutant harboring and expressing a functional Cas9 gene is obtained, it may be used to more efficiently allow integration and knockout of genes of interest in a targeted manner.



Model of Cas9 mediated double strand nuclease activity

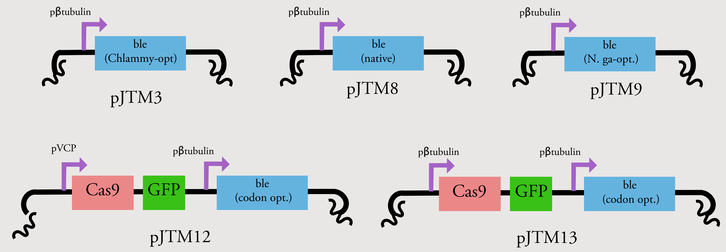
Methods

In this project we are exploring a variety of electroporation methods that may be conducive for effective transformation. We are investigating the best methods for transformation of several different strains of *Nannochloropsis*. Currently, our most effective transformations have relied on 3µg of DNA per reaction with all cell handling done at room temperature. Upon successful electroporation, the cells are subjected to 24 hours of recovery in liquid media. The cultures are plated on agar plates with zeocin included. Colonies may appear after 3-5 weeks, whereupon up to 50 colonies are picked and respotted on fresh antibiotic-containing plates. We have developed a PCR based screening method, whereby we may determine the rate of effective transformation. In these procedure, we are randomly incorporating the DNA construct into the genome of the various *Nannochloropsis* strains.



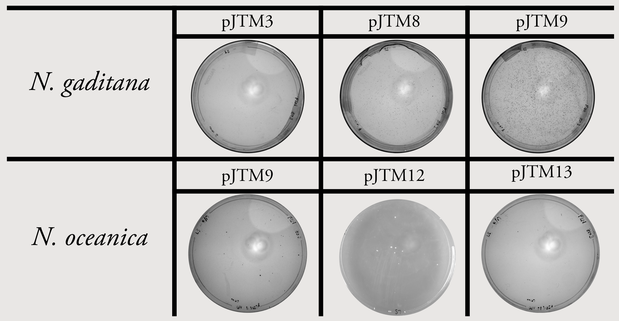
Nannochloropsis
Photo credit: Wikipedia

DNA constructs



In the optimization of transformations for *Nannochloropsis,* we are investigating the effect of codon optimizations. Additionally, we are using a combinatorial approach to investigate the effectiveness of a variety of promoters and other genetic elements. The Cas9 and GFP genes were sourced from Sigma Aldrich and the Chlammy optimized *ble* cassette was obtained from the Chlamydomonas Center. All other constructs of *ble* were synthesized in this study.

Electroporation Results

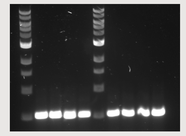


Results / Future Directions

In this investigation, we see different numbers of colonies in transformations using two different strains and several different genetic constructs. When using *N. gaditana* as a host we saw: fewer than 50 colonies with pJTM3, hundreds of colonies with pJTM8, and thousands of colonies with pJTM9. When screening transformation with pJTM3, we found that 6 of 8 colonies tested positive. We have also screened the colonies from effective transformations of *N. gaditana* with pJTM8 and pJTM9 and found that out of 50 screened colonies, 48 or more were shown to be positive through PCR screening.

When examining the most effective construct, pJTM9 with *N. oceanica*, we saw less than fifty colonies. When using the Cas9 constructs we saw even fewer colonies. Transformation with pJTM12 yielded 0 colonies while transformation with pJTM13 yielded less than 10 colonies. Upon PCR screening, all colonies showed positive amplification of the *ble* cassette.

Continuing investigation will include additional genetic constructs that feature combinations of native and synthetic promoter and terminators. Additionally, we plan to use fluorescent microscopy techniques to confirm the proper expression of chimeric *Cas9-gfp* as well as determine the localization of the fusion protein. Upon successful Cas9 integration, we anticipate targeting native endonuclease restriction sites for modification to probe the genome editing efficiency of Cas9 with restriction fragment length polymorphism (RFLP). By harnessing and optimizing this technology for genetic engineering of *N. gaditana*, we seek to augment this organism for a greater capacity to retain fixed carbon and improve biomass productivities.



PCR screening method for Nannochloropsis

Conclusions

From this study we compare the efficiency of transformation while modulating the sequences of the genetic cassettes used for the transformation of *Nannochloropsis*. We have seen that codon optimization leads to most reliable transformations in *Nannochloropsis gaditana*. Additionally, the native *ble* gene was more effective in *N. gaditana* than the *ble* gene that was optimized for *Chlamydomonas*. Through a PCR based screening method, we determine the effectiveness transformations of *N. gaditana* and found that over 96% of transformants showed positive amplification of the *ble* insert.

We are additionally investigating the differences in transformation of several different strains of *Nannochloropsis.* Using the same genetic cassettes, we have seen more successful transformation efficiencies with *N. gaditana* compared to *N. oceanica*. The physiological differences between these two highly related strains leading to such a different transformation efficiency remains an avenue of active investigation.

Acknowledgements

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