Contract No:

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Molecular Breeding Algae for Improved Traits for the Conversion of Waste to Fuels and Commodities

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Abstract

This Exploratory LDRD aimed to develop molecular breeding methodology for biofuel algal strain improvement for applications in waste to energy / commodity conversion technologies. Genome shuffling technologies, specifically protoplast fusion, are readily available for the rapid production of genetic hybrids for trait improvement and have been used successfully in bacteria, yeast, plants and animals. However, genome fusion has not been developed for exploiting the remarkable untapped potential of eukaryotic microalgae for large scale integrated bio-conversion and upgrading of waste components to valued commodities, fuel and energy. The proposed molecular breeding technology is effectively sexual reproduction in algae; though compared to traditional breeding, the molecular route is rapid, high-throughput and permits selection / improvement of complex traits which cannot be accomplished by traditional genetics. Genome fusion technologies are the cutting edge of applied biotechnology. The goals of this Exploratory LDRD were to 1) establish reliable methodology for protoplast production among diverse microalgal strains, and 2) demonstrate genome fusion for hybrid strain production using a single gene encoded trait as a proof of the concept.

FY2015 Objectives

- Establish and improve methodology for successful protoplast production in green microalgae.
- Demonstrate genome fusion and strain development using the parental strains *Nannochloropsis limnetica* (freshwater) x transgenic *N. salina* (saltwater) harboring an antibiotic resistance gene.

Introduction

Microbes account for the bulk of biological (biochemical) diversity on the planet. This natural reservoir holds tremendous potential for the molecular conversion of diverse waste forms into valued commodities, fuel and energy. Natural biological prospecting is risky however; it demands a substantial and committed investment and the probability of success is prohibitively low. Conversely, molecular breeding tools are now available to vastly accelerate the process of biological trait discovery, development, and fine tuning for applied biotechnologies. Molecular breeding is analogous to traditional breeding programs whereby sexual reproduction is used to propagate traits of interest in offspring. Here, we aimed to establish methodology that would permit molecular breeding (by a technique called protoplast or genome fusion) of micro-algal strains to produce genetic hybrid(s) to benefit commercial scaled production of algae. This technical achievement would vastly improve the financial models for large scale algae production and conversion, thus narrowing the price gap compared to conventional petroleum products.

Approach

The parental strain pairings identified for this LDRD were specifically chosen to straightforwardly enable the first demonstration of protoplast fusion for trait improvement in eukaryotic microalgae. *Dunaliella salina* was grown on modified Johnson's medium containing 30% NaCl; cells divide rapidly by asexual reproduction at high salt concentrations. *D. salina* cultures were stepped down to 2% NaCl prior to hybridization to slow cell division and induce the sexual reproductive cycle. *Nannochloropsis* strains were grown in modified f/2 medium. *Chlorella vulgaris* and *C. sorokiniana* were grown in freshwater BG-11 medium. Only the asexual reproductive cycle is known for these strains. All cultures were grown under a 16 hr - 8 hr diel cycle of full spectrum light. Cells were be harvested by centrifugation and cell wall polymers removed by chemical and enzymatic digestion to yield protoplasts. This step was elaborated as a full experimental matrix of chemical (synthetic gastric juice, mannitol) and enzyme combinations (cellulase, agarase, pectoylase, pectinase, lysozyme) and incubation conditions to maximize the yield of viable protoplasts. Efficiency and effectiveness of protoplast production was determined by specific staining of cell wall polymers and visualization by fluorescence microscopy.

FY2015 Accomplishments

- MTA was established with colleagues at Los Alamos National Laboratory and the University of Arizona to permit access to trait specific strains.
- Protoplast production was optimized for numerous different biofuel production strains of algae.
- Groundwork was laid for demonstration of genome fusion in eukaryotic microalgae.