University of Florida

Optimization of Algae Species for Biofuels Production Using Genetic Alteration

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Description: The central challenges to viable algal biofuel production are the solar energy conversion efficiency for algae growth, sustainable yields of usable products and operational constraints on production systems. While theoretical solar conversion efficiencies for algae and plants are between 5 and 6% of total insolation, most algal systems operate at average annual efficiencies well below this range. Therefore large areas are needed to produce significant amounts of biofuels from algae, and production systems must be able to sustainably produce biomass convertible to biofuels within reasonable logistical and economic constraints. Logistical constraints include minimal use of valuable freshwater and arable land resources. Economic constraints may demand the use of low tech open pond systems, rather than more costly and maintenance intensive closed bioreactor designs. Sustainability of production will depend on the ability to maintain relatively pure mass cultures of algae capable of producing high levels of desirable products (e.g. hydrocarbons or convertible lipids). These considerations point toward the need to focus on the development of systems which use ocean water and algal species adaptable to extreme conditions that minimize competition from "weed" species, such as high salinity, temperature, pH, low nitrogen availability or UV light exposure.

The focus of this study is genetic alteration of selected species of algae to optimize their performance in biomass production systems aimed at biofuels. Two approaches to genetic alteration will be explored, mutagenesis and transformation. The research program began with the use of chemical mutagens to generate altered strains of algae currently available in the culture collection of the PI (E. J. Phlips). Mutated algae are going through a selection process to identify strains with favorable characteristics. The selection criteria include growth rate, tolerance to environmental extremes (e.g. salinity, temperature, pH, UV exposure), and lipid content. The initial target species for mutagenesis research will include: 1) *Botyrococcus braunii*, a green alga (Chlorophyta) known for its high levels of hydrocarbons, but low growth rates and low adaptability to high salinities and temperatures, 2) *Synechococcus sp.* a fast growing cyanobacteria high biomass production potential, and adaptability extreme environmental conditions, such as high salinity and temperature.

Budget: $15,000
Universities: UF
Collaborators: Drs. Mathius Kirst in the University of Florida's Genetic Institute and Charles Guy in the Department of Environmental Horticulture at the University of Florida.

Executive Summary

A major challenge that the United States and the world is facing is how to produce clean, renewable energy in a cost effective manner with minimal environmental impacts. The research in this study was aimed at developing such a technology by synergistically combining blue-green algae (i.e. cyanobacteria) grown in outside ponds with anaerobic digesters to produce renewable natural gas (RNG). The research focused on a remarkable marine cyanobacterium that needs neither fresh water (grows in saline conditions) nor externally supplied nitrogen and produces large amounts of extracellular polysaccharide that can be effectively used as a substrate for RNG production. Whereas current algae-based approaches rely on producing lipids, which subsequently requires energy intensive separation processes, the approach taken here uses the algae suspension directly to produce methane gas. Furthermore, by re-cycling the

waste CO2 produced during the digestion process to enhance algae growth, a higher quality fuel gas is generated and greenhouse gas emissions are reduced.

The long-term focal points of our reseach are: 1) Selection of species with eco-physiological characteristics uniquely well suited for application in saline open pond biomass production systems, 2) Development of an innovative approach for the conversion of algal biomass into biofuels, and 3) Genetic alteration of selected species of algae to optimize their performance in biomass production systems aimed at biofuels. The species selection criteria used for the study funded by FESC in 2011/2012 included growth rate, tolerance to environmental extremes (e.g. salinity, temperature), and chemical content convertible to biofuels. For the FESC project we selected a strain of marine cyanobacteria, *Synechococcus sp* 0011, previously isolated from a coastal lagoon near the Florida Keys. This cyanobacterium (blue-green alga) grows rapidly with nitrogen obtained through nitrogen fixation and secretes large quantities of polysaccharide.

In addition to species selection, efforts were initiated to develop a process for producing methane by coupling algae production with salt tolerant methanogenic systems that we are currently operating. The use of *Synechococcus* Sp. 0011 in the production system offers the following benefits: 1) Compatibility with open photobioreactors containing saline media, thereby avoiding the use of limited freshwater resources and reducing the potential for contamination by undesirable species, 2) Secretion of energy-dense polysaccharide into the surrounding media, which can either be converted to methane without separation or recovered for use in ethanol production through a low-energy separation method and 3) Fixation of atmospheric nitrogen, thereby avoiding the use of fossil derived nitrogen nutrients.

Biomass and polysaccharide production rates of *Synechococcus* Sp. 0011 were explored using laboratory mesocosms. The focus of the study was tolerance to salinity variation, which is a critical issue is the sustainability of open-raceway production systems using marine water. The results of the mesocosm experiments demonstrate that *Synechococcus* Sp. 0011 is tolerant to salinities from 5 (near freshwater) to 70 psu (twice the concentration of ocean water). It also demonstrates an ability to adapt elevated salinities, as evidenced by the increased rates of growth with longer term exposure to high salinities.

In the Fall of 2011 we formed a new research collaboration with Drs. Pratap Pullammanappallil of the Department of Agricultural and Biological Engineering (U. of Florida), Spyros Svoronos of the College of Engineering (U. Florida) and Ben Koopman of the College Engineering (U. of Florida) to help develop a technology for the conversion of algal biomass into renewable natural gas. Preliminary results indicate that *Synechococcus* Sp. 0011 is a viable substrate for methane production, but additional research is needed to arrive at definitive conversion rates.

In the Fall of 2012 the genome of *Synechococcus* Sp. 0011 will be sequenced by Bailey Trump at the University of Florida Genetics Institute with the assistance of Dr. Mathius Kirst. This is an important step toward the next goal of genetically modifying the species to improve on its already unique properties. The availability of a sequence will also aid in the eventual patent process. In addition, genetic alteration of *Synechococcus* Sp. 0011 will be explored, using mutagenesis and genetic transformation approaches. The research program will begin with the use of chemical mutagens to generate altered strains. Mutated algae will go through a selection process to identify strains with favorable characteristics.

From a broader perspective, the research team is currently focusing on detailed design elements of the new technology. A seed grant to help the development effort was awarded to the team by Florida Sea Grant in 2012. A full proposal for future research and development is being prepared for submission in 2013.

This project has been completed. The final report can be found here.