

Flow Cytometry Technology Applied to the Characterization and Optimization of Algal Cells for Biofuel Production

Babetta L. Marrone, Taraka Dale, and Scott N. Twary, National Flow Cytometry Resource, Advanced Sciences Measurement, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545

Introduction

Algae show promise as a potential source of lipids for biofuel production; however, an understanding of the detailed biological processes of many algae as it relates to controlled lipid production is sorely lacking, and much of the basic regulatory mechanisms in algae are not well-understood. To address these issues, we are adapting flow cytometry methods commonly used in mammalian cells to the study of algal biology. While some algal flow cytometry experiments have been reported for use in the pharmaceutical and nutraceutical industries, these types of experiments have not been carefully applied in the context of the biofuel industry.

Methods

We are currently developing assays for monitoring lipid content, cell cycle progression, viability and cytotoxicity by utilizing bright field microscopy, fluorescence spectroscopy, conventional flow cytometry, and the NFCR High Resolution Spectral flow cytometer. A panel of algae has been acquired, which includes both marine and freshwater algae, as well as samples of one strain grown under putative low and high lipid-producing media condition. This panel is being analyzed in the development of these new methods as they relate to algae.

Results

Microscopy shows that lipid droplets can be readily observed, and that different samples contain cells of differing size and degrees of compartmentalization. Flow cytometry data correlates well with the microscopy in identifying strains of different sizes, different sized cells within a strain sample, and changes in size and autofluorescence due to putative lipid production. In addition, the bulk fluorescence of the samples has been measured, and strains have been analyzed using the High Resolution Spectral flow cytometer to determine the individual fluorescent spectra of algal cells in each sample.

Conclusions

These assays will be refined with additional samples, and an expanded set of conditions designed to vary the lipid content and general growth of the different strains will also be assayed. Furthermore, lipid or membrane dyes will be used to identify differences in lipid content and/or composition between strains and conditions. Lastly, we plan to use cell cycle analysis to correlate algal growth with lipid production. After our methods are established, we will demonstrate that our methods detect the expected effects based on culture/environmental conditions. Then the assays will be utilized to determine differential protein expression in high and low lipid-producing algae. The above studies will allow us develop strategies for monitoring and optimizing biorefinery operations; for discovery and screening of new strains with attractive characteristics; and for identifying and isolating subpopulations of interest for further study.

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