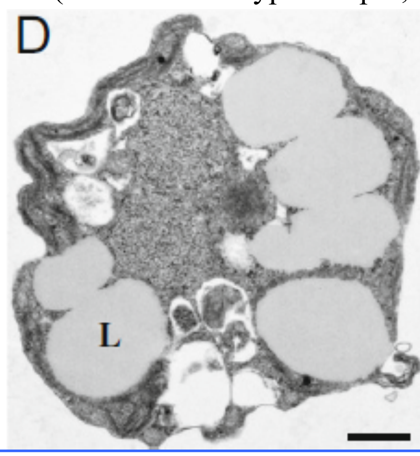
Sandia Algal Research Wins Cover of September 2012 *Biotechnology and BioEngineering*

Challenge

Many species of algae—both eukaryotic (plant-like more-complex cells) and prokaryotic (bacteria-like simpler cells)— produce lipids (fats), using energy from the sun in photosynthesis. In simplified terms, solar energy "activates" carbon dioxide (CO_2) and water in a complicated set of biochemical reactions (partly known as the Calvin Cycle), the result being the (photo)synthesis of bio-organic molecules—carbohydrates, proteins lipids, and DNA/RNA. Some of these molecules are necessary for cellular housekeeping functions and cell growth and division to produce new algal cells. But the accumulation, inside the cell of lipids that are readily convertible to biodiesel is a feature that is the subject of intense study. This LDRD-funded, Truman Fellowship Project by Anne Ruffing focused on genetically engineering the prokaryotic alga (also called cyanobacteria) *Synechococcus elongatus* to increase its production of biofuel precursor lipids. In addition to optimizing the amount of lipid produced by the algal cells, a second important question addressed by the research was the impact of optimizing lipid biosynthesis on the remainder of the algal cell's metabolism and physiology.

Research

Using genetic engineering techniques, the project team created numerous genetic variants designed to shift the cell's metabolism toward increased free fatty acid (FFA) production. Because their chemical structures allow them to be converted in one fairly simple reaction step, free fatty acids are a quite convenient biodiesel precursor once they are excreted by the algae into their surrounding environment. (FFAs are one type of lipid, steroids like cholesterol and estrogen are another type.)

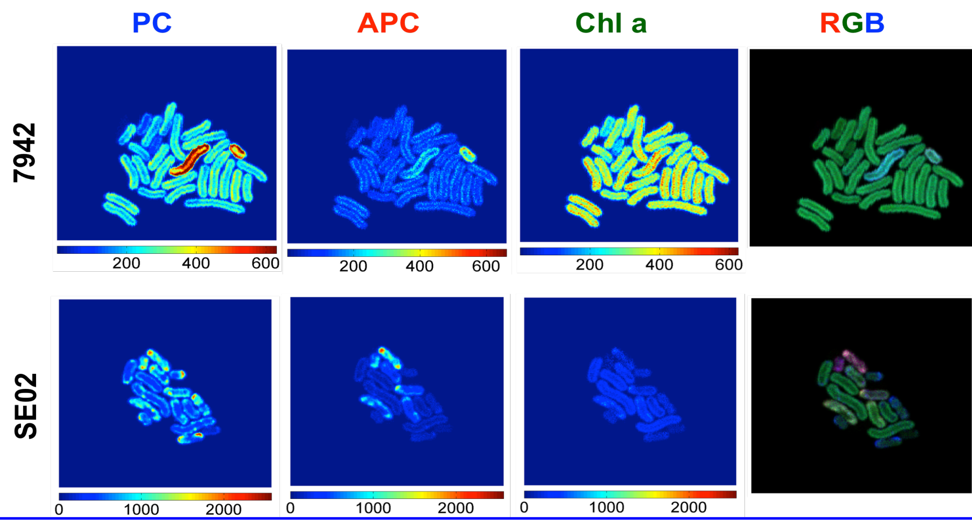


Accumulation of Lipid (L) inside an Algal Cell

Certain secondary alterations observed in the cells' metabolism made physiological "sense." For example, the cells increased their expression of the genes encoding the information for FFA transporters, proteins that facilitate movement of FFAs from inside the cell to its outside environment. Logically, this would be expected as a positive genetic/metabolic adaptation—if the cells are synthesizing extra FFAs, they must have an increased ability to export that excess.

Unfortunately, not all the news trended positive. For example, certain algae genetically engineered for higher levels of FFA synthesis/export showed reduced overall cell growth coupled with a decrease in photosynthetic capabilities. In turn, decreased photosynthesis was coupled to negative changes in the quantity and location of chlorophyll a in the cells. In addition to making algae and plant cells appear green, chlorophyll harnesses solar energy, converting it to chemical potential energy, that is, the energy required to suture together carbon dioxide molecules to form larger organic molecules such as FFAs. Logically, diminution of chlorophyll would be expected to be correlated with slower cell growth, since less solar energy would be used to drive the synthesis of biomolecules from CO_2 and H_2O.

Measurements were made utilizing Sandia's capabilities in hyperspectral imaging, and the work was done in collaboration with LANL and the Kansas Lipidomics Research Center.



Imaging of photosynthetic pigments in two different genetic types (strains) of Synechococcus algal cells. Chlorophyll a is shown in the third panel from left in each strain.

Significance

First and foremost, the research demonstrated multiple genetic manipulations that can be used to engineer prokaryotic algae (cyanobacteria) for enhanced FFA production and excretion (transport to the external environment). This manipulation and utilization of the simpler cyanobacteria may be quite useful, given that their nutrient requirements are less demanding that that of eukaryotic algae, and that they are easier to genetically manipulate. In addition to identifying these genetic targets for engineering initiatives, concomitant identification of potential side-effects such as slower cell growth provides subsequent researchers with key knowledge about the most desirable physiological changes to yield optimal FFA production for biofuels.
Considering the importance of low-carbon transportation fuels for the near term—until enough alternative energy electricity generation and new battery technology can favor affordable electric vehicles—biodiesel from algae is positioned to play a key role in powering transportation vehicles.

The results of this project earned the cover story in the September 2012 issue of the journal *Biotechnology and Bioengineering*

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