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Effects of Lipid Activating Chemical Compounds on the Growth and Production of Fatty Acids and Metabolites in Green Algae

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Abstract

This study examines the effects of small molecule lipid activating chemical compound, discovered from the high throughput screening (HTS) (2) methods of previous FATTT Lab studies on the growth and production of lipids and metabolites in microalgae. For this study, the effects of two lipid inducing chemical compounds from the HTS method were implemented with the microalgae strain Chlamydomonas reinhardtii CC-125. This experiment focused on scaling up to large amounts of culture, approximately 1L. These cultures were grown in specially designed large bioreactors that were able to accommodate for such large volume of algal culture. Algal cells were treated with 10 µM concentration of the chemical compounds at initial time of inoculation. A control set was also implemented to be compared against the treatment conditions. Daily samples of algae cultures were taken in order to analyze growth on a time course-based method in addition. After five days, algae cultures were harvested completely, spun down into pellet form to be freeze dried vacuum by lyophilizer machine. The dried biomass was then recorded and used to carry out analytical techniques to quantify total lipids and metabolites of the algal cells.

Introduction & Methods

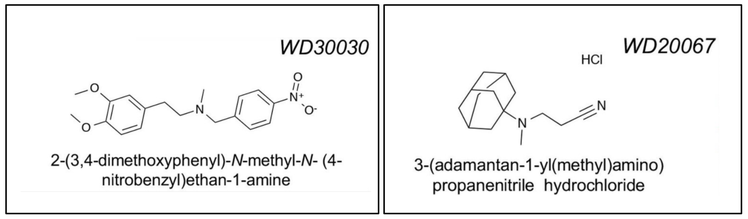
Introduction

Green algae have been on the rise as a favorable alternative source in biofuel research. Because of their unique structure, green algae can produce and store high amounts of lipids and fatty acids, which makes them an ideal biofuel alternative. Green algae will produce high amounts of lipid when they are in a nutrient stressed environment that is either lacking certain ions, like nitrogen, or other factors. Algal cells in the nutrient stressed environment can become chlorotic. High throughput screening and discovery of lipid inducing small molecules (2) from previous UNL FATTT Lab studies are being implemented in current algal research to increase production of lipids and growth without the need of nutrient starvation/deprivation or severely reducing biomass of algal cells (2).

Methods

Stock cultures of CC-125 were grown in Tris-Acetate-Phosphate (TAP) media in 100mL volume under white fluorescent light at 25C and left on rotary shaker. Once enough stock cultures was acquired, these were spun down into pellet form to create a more concentrated density stock solution to inoculate experimental samples. Bioreactors of 1000mL volume were filled with 1000mL of TAP media. Compounds WD5345030 and WD5234067 were added to each set of bioreactor tank to achieve a concentration of 10 µM of compound, in this case 1mL of compound was added to each tank. A control set was also run with the experimental set. In the experimental set, algal cells were added with 1mL of the vehicle dimethyl sulfoxide (DMSO) for a concentration of 10 µM. Control and experimental conditions were run in duplicates. The experiment life was ran for 5 days total. Each day, small amounts of culture were taken from each set to carry out further analytical measurements. 5mL from each tank were extracted and spun down into pellet form, supernatant removed, and stored in -80C freezer. Another 1mL of culture was extracted to perform optical density and Nile Red readings. Of the 1mL sample, 200 µL of culture were placed in 96 well plate tray in duplicate samples. A blank of just TAP media was added in the last column. Algal cells were then treated with the lipophilic dye Nile Red which attaches itself to lipid accumulation in algal cells. 5 µL of Nile Red of 10mM concentration were added to each well. These were then covered in foil and placed in 32C warm oven for 30 minutes. The well plate was then shaken and then read in a microplate reader using the GenSec 5.0 program. The program takes two scans, one at OD600 and a fluorescent scan for the Nile Red treatment. On the third day of the experiment, half (500mL) of the culture was harvested, supernatant removed, and lyophilized. On the 5th day, all cultures were spun down again, supernatant removed, and lyophilized. All dry biomass was then used in analytical techniques to quantify data on lipids and metabolites. Lipid and metabolites were extracted using the Bligh and Dyer Lipid extraction(1) and Domingo-Almenara et. al. Quantification and identification of metabolites in GC/MS (2), respectively. 20 mg of each biomass was used in each analytical processing in order to acquire enough sample for both lipid and metabolite analysis.

Lipid Activating Chemical Compounds



Picture Source: (2) Wase, N., Boqiang, T., Adamec., J., Cerny, R., Black, P., DiRusso, C. (2014). Discovery of small molecule hyperlipid accumulating triggers of algae Chlamydomonas reinhardtii identified via in vivo phenotype based screen. The Federation of American Societies for Experimental Biology Journal, 28(1) 579.1

References

(1) Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917. doi:10.1139/o59-099

(2) Domingo-Almenara X, Brezmes J, Vinaixa M, Samino S, Ramirez N, Ramon-Krauel M, Lerin C, Diaz M, Ibanez L, Correig X, Perera-Lluna A, Yanes O (2016) eRah: A Computational Tool Integrating Spectral Deconvolution and Alignment with Quantification and Identification of Metabolites in GC/MS-Based Metabolomics. Anal Chem 88: 9821-9829

(3) Wase, N., Boqiang, T., Adamec., J., Cerny, R., Black, P., DiRusso, C. (2014). Discovery of small molecule hyperlipid accumulating triggers of algae Chlamydomonas reinhardtii identified via in vivo phenotype based screen. The Federation of American Societies for Experimental Biology Journal, 28(1) 579.1

(4) Wase, N., Boqiang, T., Allen, J. W., Black, P. N., DiRusso, C. (2017). Identification and metabolite profiling of chemical activators of lipid accumulation in green algae. Plant Physiol. 174, 00433.02017. doi: 10.1104/pp.17.00433

Results

Figure 1 - Physical Changes In Bioreactors Overtime

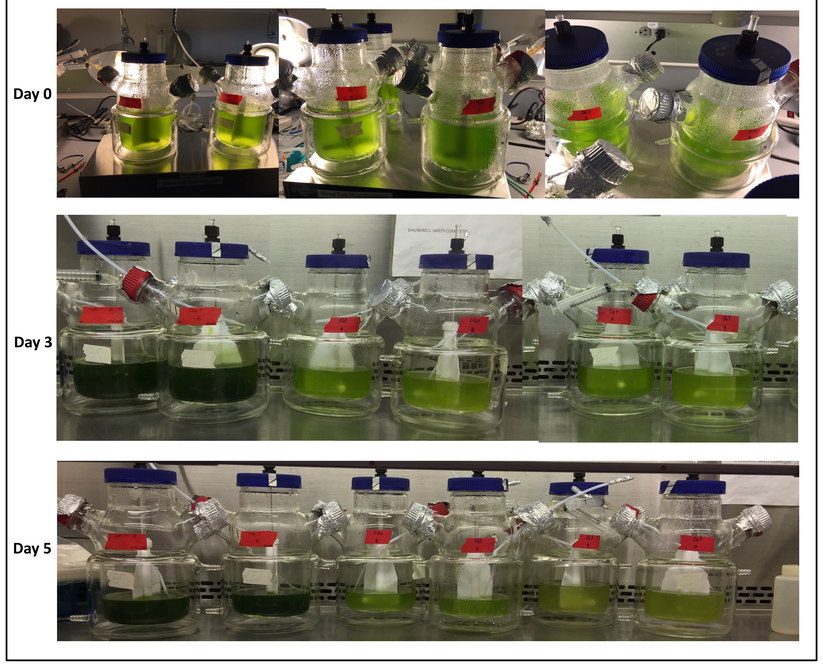


Figure 1 – Physical Changes in Appearances of Bioreactors from Day 0, Day 3, and Day 5 Bioreactors from Left to Right: Control A , Control B, 030-A, 030-B, 067-A, 067-B.

Figure 2A - Growth of OD600 nm

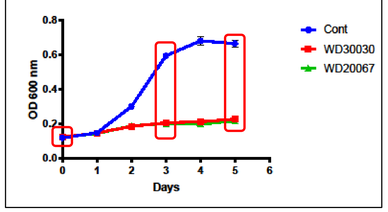


Figure 2B - Growth of Algal Dry Weight

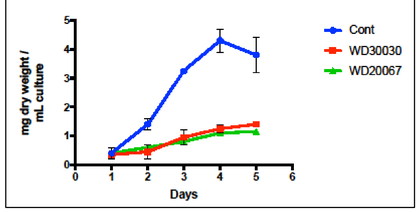


Figure 2A – Growth of algal cells were measured by taking optical density measurements at 600nm wavelengths overtime in microplate reader. OD600 readings were read on 96-well plates with 200µL aliquots of culture. Red bars indicate specific day in which algal mass was taken to perform metabolite analysis. Figure 2B – Growth of algal cells were measured by weighing out dried biomass after each 24 hour harvest time. 5mL of algal culture were taken each day for dry weight growth.

Figure 3 – Nile Red (lipid productivity)

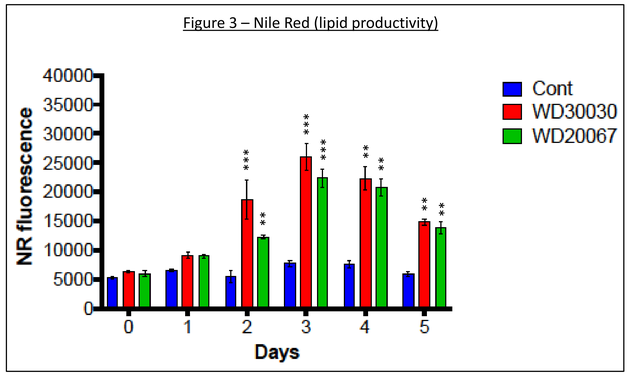


Figure 3 – Nile Red fluorescence measured over time. 200µL of algal culture were placed in 96-well plate aliquots and treated with 5 µL of lipophilic Nile Red dye before being scanned for fluorescence. * represent levels of significance.

Metabolite Analysis

Figure 4 – GCMS Profile

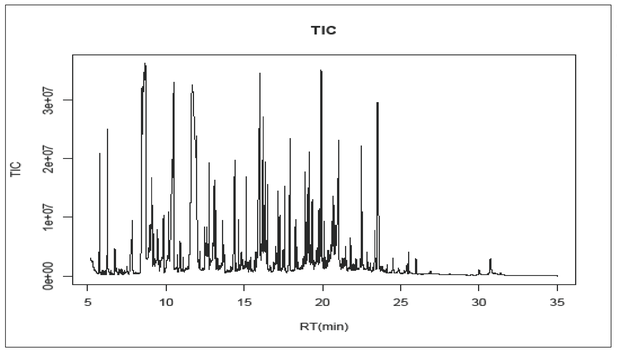


Figure 4 - Gas chromatography mass spectrometry profile figure of metabolite analysis showing total ion count.

Figure 5 - Principal Component Analysis of Compounds and Time Duration on Metabolism

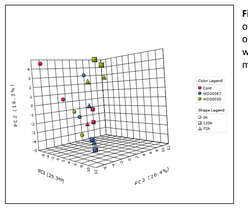


Figure 5 – Principal component analysis of metabolites which displays the effect of control and compound treatments with time duration and their effect on metabolism.

Figure 6A – Glycine Change Overtime

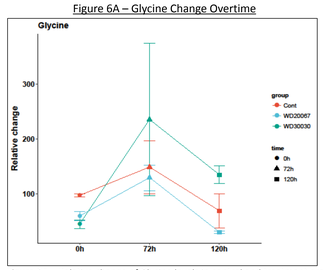


Figure 6B – Glycine Profile Comparison and Match Factor

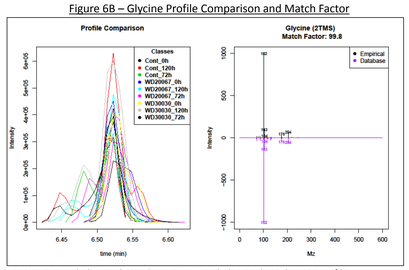


Figure 6A – Relative change of Glycine levels in control and treatment groups overtime in metabolite analysis. **Figure 6B** – Metabolite analysis showing profile comparison of Glycine peak intensity to other treatment conditions along with the corresponding match factor analysis using NIST Library spectra of glycine.

Figure 7 – Heatmap of Metabolites

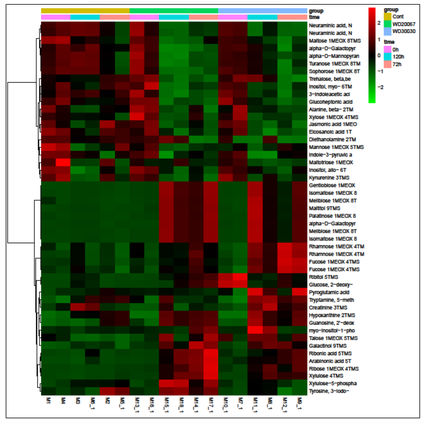


Figure 7 – Heatmap displaying the expression of significantly changed metabolites in metabolite analysis of control and treatment groups over 0, 72, and 120 hours.

Conclusion

- The study conducted is still a work in progress and the current data/information shown is only preliminary data. Replication of experiment will still need to be performed in order to additionally confirm result findings here.

Future Directions

- Analytical analysis of lipid content in algal biomass using FAMES (1) method to identify different types and levels of lipid still need to be performed.
- Repeat of experiment testing different lipid activating chemical compounds from high throughput screening.
- Repeat of experiments to confirm data.

Acknowledgments

Special thanks to the entire FATTT Lab for supporting and assisting with the research project. Thank you UCARE program for the grant and opportunity to do research.