CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

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Project Number

S1997

Project Title

Developing Screening Conditions to Identify Fatty Acid Export Mutants in Chlamydomonas reinhardtii

Abstract

Objectives/Goals

The objective of the project is to develop conditions that can be used to identify C. reinhardtii individuals that are unable to export fatty acids from the plastid to the endoplasmic reticulum. Fatty acid export is an important step in the synthesis of the triacylglycerol (TAG) molecules used to generate biofuels. The screen can be used in conjunction with a knockout approach to identify the genes underlying this pathway in order to contribute to ongoing efforts to explore biofuels as an economical form of alternative energy.

Methods/Materials

Spectrophotometry was used to track optical density (OD) of cultures grown in TAP (Tris-Acetate-Phosphate) minimal medium and medium supplemented with the detergents NP-40 and Tween 80. At the last time point, 200 cells from each culture were transferred to agar plates to observe viability. Thin-layer chromatography was used to confirm that detergent improved fatty acid distribution in water-based medium. Growth in cultures treated with cerulenin (a fatty acid synthase inhibitor) that were grown in either fatty acid-supplemented medium or minimal medium was compared using OD.

Results

Cultures grown in the presence of either detergent showed significantly higher growth rates than the control, and viability was almost three times greater. Although TLC results were inconclusive as to whether detergent evened the distribution of fatty acids in water-based medium, the quantity of emulsified fatty acid was increased. When cerulenin-treated cells were then grown in detergent-supplemented medium, their growth was dramatically decreased in the control condition but returned to slightly less than pre-treatment levels when exogenous fatty acids were supplied.

Conclusions/Discussion

The data produced in this experiment demonstrates that fatty acid pathway-deficient C. reinhardtii can be rescued by exogenous fatty acids. Once the genes involved in the export pathway are identified, the scientific community will be able to upregulate their expression, boosting TAG output in this algal species.

Summary Statement

I developed screening conditions to find C. reinhardtii cells that are deficient in the fatty acid export pathway; this result can be used with a knockout experiment to identify genes important for biofuel research.

Help Received

Facilities for experimentation were provided by the Jonikas Lab at the Carnegie Institution at Stanford University. Dr. Martin Jonikas provided advice, and Dr. Xiaobo Li provided training and guidance on the project.