Project Proposal

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Introduction

Chlamydomonas reinhardtii is a unicellular alga that is well established as a model system. Like other eukaryotic microalgae, *C. reinhardtii* exhibits substantial triacylglycerol (TAG) accumulation under certain stresses, such as nitrogen deprivation conditions. TAG is of interest because it is an essential precursor for biofuel production.
Glycerol-3-phosphate dehydrogenase (GPD) is an enzyme that catalyzes the synthesis of Glycerol-3- phosphate, an indispensable precursor for glycerolipid synthesis and the accumulation of TAG in eukaryotic microalgae. In *C. reinhardtii*, five GPD homologs have been found (Morales-Sánchez, Kim, Terng, Peterson, & Cerutti, 2017). Among them, GPD2 homolog is abundantly expressed under nutrient deprivation conditions and osmotic stress. When the expression of GPD2 is decreased, TAG and glycerol production decrease significantly in nitrogen- starvation and high-salinity conditions respectively(Morales-Sánchez et al., 2017). Based on these observations, we hypothesize that GPD2 homolog contributes to the catalysis of both compounds: glycerol and TAG, depending on the environmental condition.
In addition, GPD2 has been located in the chloroplast. This suggests a novel plastidic pathway for glycerol production under high salinity conditions. Another goal of my research is to elucidate this chloroplastic pathway for glycerol production which could involve specific pathways for glycerol synthesis and removal during the osmoregulatory process.
Finding how cells regulate GPD2 enzymatic activities, what pathway(s) this enzyme plays a role in, and what other enzymatic components GPD2 may interact with, can help to broaden our biochemical and cytological understanding of TAG and glycerol synthesis and accumulation in the cell, with possible implications for biotechnology.

Objectives

1. To learn how to analyze obtained data and generate publication-quality graphs using R programming language.

2. To learn how to perform metabolomics analyses using R by utilizing publicly available data and carrying out 'practice' analyses based on these data.

Methods

For objective 1, datasets were collected as part of ongoing research. For this group of experiments *C. reinhardtii* cells were stressed with 100 mM of NaCl for 6 hours and glycerol production was measured. Glycerol is an important osmoprotectant in *Chlamydomonas* and it increases under osmotic stress conditions. GPD2 overexpressing cell lines (OX), anteriorly created in our lab, were used to investigate the proposed hypotheses. Glycerol production was estimated using free glycerol reagent. Results show that OX cell lines are producing glycerol even when ribosomal protein translation has been inhibited. This may indicate that already existing GPD2 proteins are catalyzing glycerol production, and this function has been triggered by some type of regulatory mechanism (such as a PTM). To try to determine how the activities of GPD2 are regulated, or what PTM is responsible for this regulation, cells have also been

treated with diverse compounds such as kinase inhibitors, phosphatases inhibitors, ROS scavengers, and others.
Additionally, there are two other genes in *Chlamydomonas* that may be important for osmotic stress adaptation: Glycerol kinase and *AKR3* genes. With the goal of determining the roles of these genes in the glycerolipid metabolic pathway of *Chlamydomonas reinhardtii*, mutants strains for *AKR3* and glycerol kinase were obtained from the Chlamydomonas Resource Center. qRT-PCR analyses were performed in these mutants and metabolomics analyses are going to be completed. For objective 2, to learn how to analyze metabolic data, practice analyses will be carry out utilizing publicly available dataset obtained by Tietel, Wikoff, Kind, Ma, & Fiehn, 2019. For their analyses, WT *Chlamydomonas* cells were exposed to hyperosmotic stress (0.5 M sorbitol). Primary metabolites were extracted and processed using gas chromatography/time-of-flight mass spectrometry (GC-TOF MS). Data was analyzed using MetaboAnalyst web server.

Conclusion

Experiments have been performed aiming to answer our research questions and testing our hypotheses, the goal of the project here proposed is to re-analyze acquired data and create quality graphs using R programming language. A second goal is to learn how to analyze data (using R) for experiments that will be performed in the near future.

References

Morales-Sánchez, D., Kim, Y., Terng, E. L., Peterson, L., & Cerutti, H. (2017). A multidomain enzyme, with glycerol-3-phosphate dehydrogenase and phosphatase activities, is involved in a chloroplastic pathway for glycerol synthesis in Chlamydomonas reinhardtii. *The Plant Journal*, *90*(6), 1079–1092. https://doi.org/10.1111/tpj.13530

Tietel, Z., Wikoff, W. R., Kind, T., Ma, Y., & Fiehn, O. (2019). Hyperosmotic stress in Chlamydomonas induces metabolomic changes in biosynthesis of complex lipids. *European Journal of Phycology*, 1–19. https://doi.org/10.1080/09670262.2019.1637547