Modeling carbon metabolism of the diatom *Phaeodactylum tricornutum* during nitrogen starvation and during high light and low light conditions

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The diatom *Phaeodactylum tricornutum* (Pt), a model photosynthetic eukaryotic microbe, has the ability to accumulate up to 45% of dry cell weight as triacylglycerol (TAG), a neutral lipid and precursor to biodiesel[1]. To take advantage of this innate ability, we need to understand how metabolic pathways adjust to changing environmental conditions. The long-term goal of this project is to promote efficient production of high-value and fuel-related compounds through optimization of metabolic fluxes in Pt. Building upon our expertise in ^{13}C metabolic flux analysis (MFA),[2] our current goal is to develop novel experimental protocols and data analysis workflows to enable ^{13}C flux analysis of Pt. We are currently investigating the metabolic adjustments of Pt to three variables, *i.e.*, light, nitrogen availability, and genetic knockout of TAG degradation enzymes, which strongly impact cell growth and lipid accumulation.

In our first study, we varied the intensity of light supplied to the Pt culture. We compared metabolic fluxes inside wild-type (WT) Pt cells grown under low-light (60 µE m^2 s^{-1}) or high-light (250 µE m^2 s^{-1}) conditions. We compared the metabolic measurements and net fluxes in these two conditions using ^{13}C metabolic flux analysis. We demonstrated that carbon was fixed at a faster rate under the high light conditions compared to low light conditions, and the cell mass composition and TAG profiles were different between the two conditions. We aim to compare the flux maps to understand how photosynthesis activity affects central carbon metabolism and TAG accumulation in Pt.

In a second study, we investigated metabolic fluxes inside wild-type Pt and a nitrate reductase (NR) knock-out strain in response to changing nitrogen availability in the culture medium. We studied three cultures, *i.e.*, Pt-WT with nitrate (WT/N+), Pt-WT without nitrate (WT/N-), and Pt-NR with nitrate (NR/N+). We found WT/N− accumulated much higher levels of TAG and total lipids than that of WT/N+, which is consistent with previous studies. Meanwhile, the chlorophyll, protein content and free amino acid levels inside WT/N− cells dropped substantially relative to that in WT/N+. In contrast, the carbohydrate content, urea and metabolites in the TCA cycle of WT/N− cells increased dramatically compared to that in WT/N+. Our results are consistent with previous findings that genes associated with urea cycle are upregulated while

expression of urea-degrading urease is downregulated in WT Pt cells under nitrogen starvation conditions. Interestingly, although NR/N+ showed a biomass composition similar to that of WT/N−, its carbohydrate content was about 50% higher than that of WT/N−. ^{13}C-labeling and targeted metabolomics revealed that NR/N+ cultures maintained smaller metabolite pool sizes in the TCA cycle and nitrogen assimilation pathways but exhibited higher labeling rates compared to WT/N−. Our ^{13}C-MFA results have revealed remarkable differences in the metabolic fluxes between WT/N+, WT/N- and NR/N+.

In a third study, we aimed to characterize metabolic changes in an acyl-CoA dehydrogenase knockout (ACAD-KO) Pt strain. When Pt cultures are switched from nitrogen-depleted to nitrogen-replete media, WT cells rapidly degrade the accumulated TAG while ACAD-KO cells retain their TAG stores. Comparing the ACAD-KO strain to WT after nitrogen repletion, we observed increases in TCA cycle labeling in the ACAD mutant. We hypothesize that the TCA cycle in the WT strain is being fed by the breakdown of the TAG, resulting in lower labeling.

Our findings based on ^{13}C MFA will help us to understand how Pt metabolism adapts to various environmental conditions and genetic modifications, which will guide strain engineering efforts to maximize TAG biosynthesis in Pt. We will conduct future MFA studies of Malic Enzyme overexpression will further our understanding of TAG accumulation and the facilitative role of cross compartment redox shuttling through modification of a central metabolic enzyme, in diatoms.[4]

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