Title : Trophic switching rewires mitochondrial–chloroplast energetics, redox balance, and ROS physiology in *Euglena gracilis*

Author(s) :

1. Sutthiphat Sriwari — **Student ID :** 650510302
2. — **Student ID :**
3. — **Student ID :**

Major : Biology

Advisor(s) :

1. Lecturer Dr. Sahutchai Inwongwan
2. Lecturer Dr. Sitthisak Intarasit
3.

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ABSTRACT

Euglena gracilis is a metabolically versatile alga capable of surviving under diverse trophic conditions, reflecting the adaptability of its mitochondrial system to different energy sources and forms. This study investigates the mitochondrial physiology of *E. gracilis* cultured in Hutner's medium under three trophic conditions: photoautotrophic (light without an external carbon source), mixotrophic (light with 1% v/v ethanol), and heterotrophic (dark with 1% v/v ethanol). Key physiological parameters, including growth, mitochondrial mass (MitoTracker GreenTM, MTG), chlorophyll content, total and specific reactive oxygen species (ROS), energy cofactors, and redox cofactors, were analyzed. Cells cultured under mixotrophic conditions exhibited the highest growth performance, with a specific growth rate of 0.71 day^{-1}, followed by heterotrophic conditions (0.68 day^{-1}), whereas photoautotrophic cultures showed the slowest growth, with a specific growth rate of 0.28 day^{-1}. MTG staining showed the strongest mitochondrial signals under heterotrophic and mixotrophic conditions, whereas photoautotrophic cells exhibited the weakest intensity. In contrast, chlorophyll signals were preserved in photoautotrophic and mixotrophic cultures but were markedly diminished under heterotrophic conditions. Total ROS and hydrogen peroxide (H_2O_2) levels were significantly higher in photoautotrophic cells and lowest in heterotrophic cells, although oxidative damage was detected across all trophic conditions. Redox cofactor analysis showed that NADP(H) pools were highest under

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photoautotrophic conditions and lowest under mixotrophic conditions. In contrast, NAD(H) pools were highest under mixotrophic and heterotrophic conditions and lowest under photoautotrophic conditions. adenylate profiling revealed that ATP, ADP, and AMP concentrations were highest in heterotrophic cells, while photoautotrophic and mixotrophic cultures exhibited comparable levels. However, the ATP/ADP ratio and cellular energy charge were highest under photoautotrophic conditions and lowest under mixotrophic conditions. the results reveal that *E. gracilis* exhibits pronounced metabolic plasticity, reallocating redox and energy pathways across trophic environments to coordinate growth, oxidative physiology, and cofactor regulation.

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