Role of chlororespiration in the high CO_2 tolerance of unicellular red alga *Galdieria sulphuraria*

Belitz Alisa Marie（筑波大学 生物学類）　　指導教員：蓑田　歩（筑波大学 生命環境系）

Introduction

While CO_2 is essential for photosynthesis, the growth of most photosynthetic organisms is inhibited when CO_2 levels surpass 2-5 percent (Solovchenko and Khonzin-Goldberg 2013). A few algal species have been reported to tolerate higher CO_2 levels, including the unicellular red alga *Galdieria sulphuraria*. *G. sulphuraria* is isolated from acidic hot springs and has been reported to grow in up to 100% CO_2 conditions. However, the mechanism of high CO_2 tolerance in *G. sulphuraria* is unknown.

In a previous study by our group, *G. sulphuraria* was suggested to optimize the light-dependent reactions of photosynthesis by a novel process that prevents the overreduction of the plastoquinone pool under high CO_2 conditions (Ozeki 2021). Transcriptome analysis indicated the upregulation of a type II NADPH dehydrogenase (NDA2) and a plastid terminal oxidase (PTOX) under high CO_2 in *G. sulphuraria*. In green algae, NDA2 and PTOX have been reported to be involved in chlororespiration, the reduction of oxygen using electrons from the plastoquinone pool, which can serve as an alternative electron outlet for alleviating excess reducing power (Saroussi et al. 2023). Thus, NDA2 and PTOX were identified as candidate enzymes for optimizing electron transport in *G. sulphuraria* under high CO_2 conditions. This research aims to investigate the role of NDA2 and PTOX in the tolerance of *G. sulphuraria* to extremely high CO_2 levels.

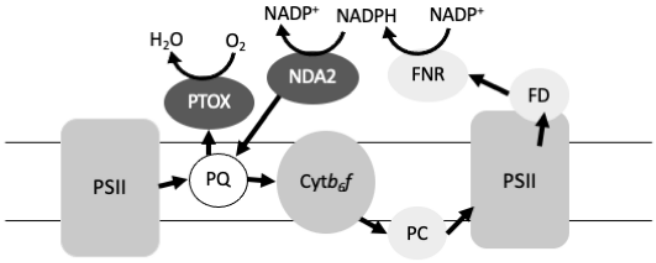

Fig.1 Schematic representation of the role of NDA2 and PTOX in the photosynthetic electron transport chain.

Materials and Methods

Culture: G. sulphuraria cells were grown at 40℃ in 2 x Allen's medium adjusted to pH 2.5 or pH 1 for diploid or haploid cells, respectively. Cells were bubbled with air or 100% CO_2 and exposed to continuous light (90±3 μmol photons m^{-2} s^{-1}). Cell growth was evaluated by OD_{750}.

O_2 electrode: Respiration rate of cells was measured at 40℃ using Oxytherm (Hansatech).

Results and discussion

1. Construction of NDA2 and PTOX knockout mutants

1.1 Isolation of haploid cells

Diploid *G. sulphuraria* are surrounded by a thick cell wall that prohibits genetic modification. Cell wall-less, haploid cells of *G. sulphuraria* were induced by decreasing the pH of culture media as described by Hirooka et al (2013). Isolated cells were used for transformation.

1.2 Genetic modification

The coding regions of the target NDA2 or PTOX genes along with the approximately 1 kbp upstream and downstream regions were amplified by PCR from genomic DNA. The coding regions were replaced by linear DNA containing a blasticidin S (BS) selectable marker. The resulting DNA was used for transformation by homologous recombination using a polyethylene glycol (PEG)-mediated method. As selection by BS has not yielded positive transformants, we are examining transformation and selection conditions. After successful transformation, we plan to evaluate the phenotype under high CO_2 conditions.

2. Effect of PTOX inhibition

Propyl gallate (PG) is known as a PTOX inhibitor. The effective concentration of PG for *G. sulphuraria* was determined due to a lack of previous reports. Growth rate of cells decreased in the presence of 1 mM PG in either air or 100% CO_2 conditions. This concentration had no immediate effect on respiration rate and was consistent with that used in studies of PTOX using green algae (Saroussi et al. 2023). We plan to measure the chlorophyll fluorescence of cells with and without the addition of PG using PAM to evaluate the effect on photosynthetic electron transport. Results will be discussed in the oral presentation.

References

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