Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Photosynthetic and physiological adaptations to stress in *Dunaliella salina*

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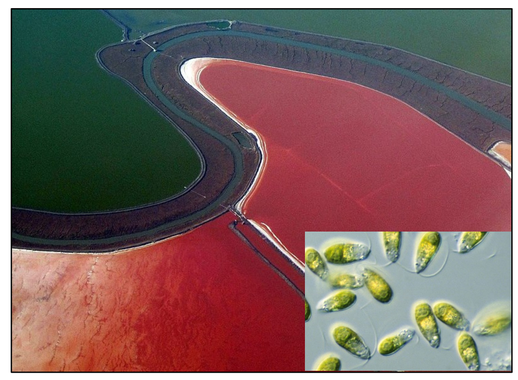
Background and Aim

Dunaliella salina is a halotolerant Chlorophyta and primary producer in hypersaline lagoons and lakes. *D. salina* is able to adapt to rapid shifts in salinity and high irradiances by accumulating more than 50% of its dry weight in glycerol, that serves as an osmoticum, and up to 10% of its dry weight in β-carotene for photoprotection (Monte, et al., 2020). *D. salina* is intensively cultivated for the production these two compounds, the production of which can be improved by a deeper physiological knowledge.

The aim of this PhD project is the characterization of the **physiological and photosynthetic responses** of *D. salina* to changes in light, salinity and sulphur availability through molecular and physiological assessments. In particular, as photosynthesis drives and limits growth in algae, the study of species with specific adaptations can reveal novel regulatory mechanisms that can improve the productivity of *D.* salina cultivations and can be applied to other species.



(Wikimedia commons)

Results

In vivo Chlorophyll fluorescence analysis

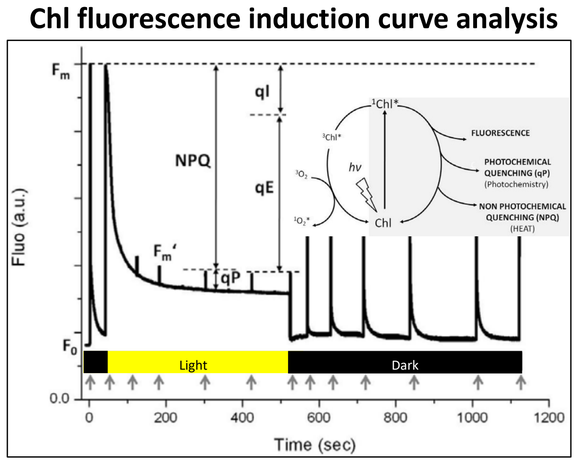
Through ***in vivo* Chlorophyll (Chl) fluorescence** analysis on dark acclimated samples, we can assess photosynthetic performance.

When light is harvested by Chls, it can undergo three fates:

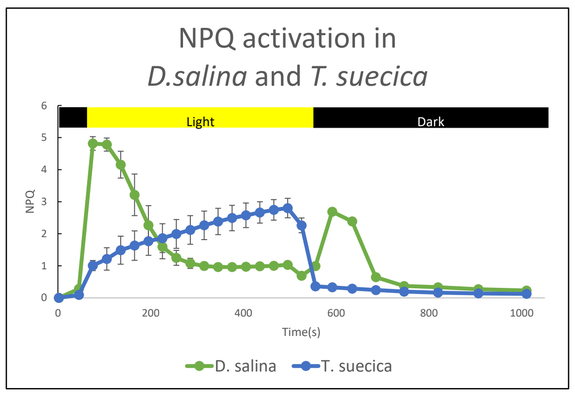
- Drive photochemistry
- Chlorophyll fluorescence
- Dissipation as heat through Non-Photochemical Quenching

Non-Photochemical Quenching (NPQ) mechanisms are activated by plants and algae to dissipate excess energy, that would otherwise damage the photosystems, as heat. *Tetraselmis suecica* displays a NPQ response similar to that of most plants and algae, suggesting the underlying molecular mechanism is the same.

Chl fluorescence induction curve analysis



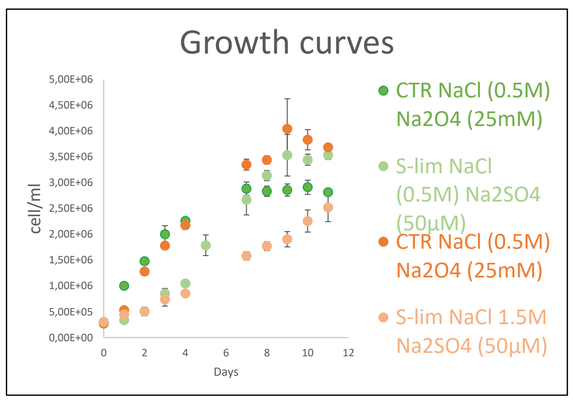
NPQ activation in *D.salina* and *T. suecica*



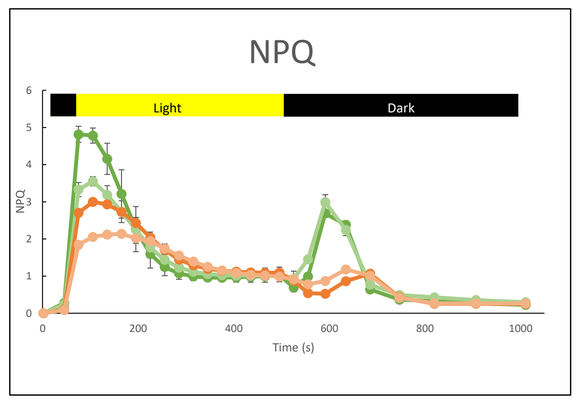
By comparison, *D. salina* displays an unusual response with what seems a peak of NPQ activity both in the light and in the dark phase, suggesting a different regulation of photosynthetic light reactions. We are performing different experiments to better characterize *D. salina* photosynthesis.

Impact of salinity and sulphur availability on photosynthesis

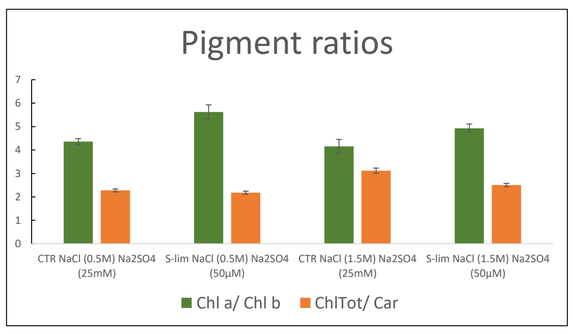
S is an essential macroelement for cells, and some elements of the photosynthetic apparatus (e.g., PSI harbours Fe-S clusters).



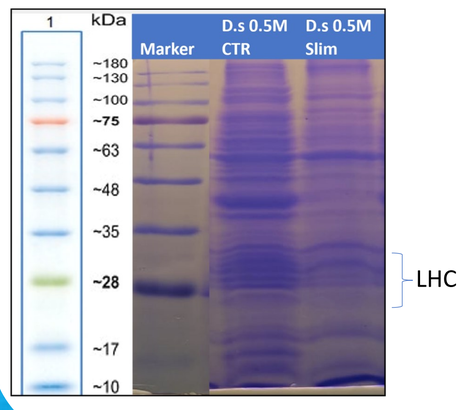
Growth curves of *D. salina* show that S limitation causes an initial lag phase in the growth.



S limitation doesn't influence NPQ activation, while salinity seems to have a greater effect.



Pigments ratios indicate higher Chl a/b ratios in S-lim conditions, suggesting the presence of less PSII antennae. While in higher salinity the Ch a/b ratio is lower but there is a higher production of carotenoids(Car).



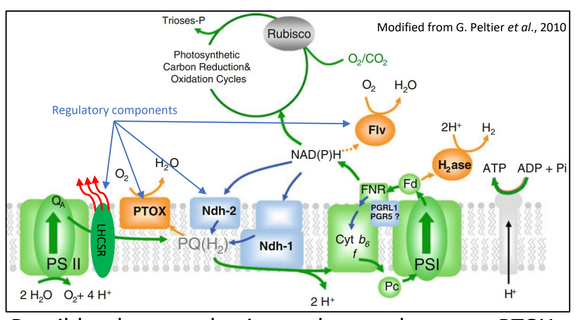
SDS-PAGE total protein separation suggests that there is a difference between control and S-lim conditions, including a modulation of antenna proteins.

Ongoing experiments:

- *Thylakoid purification for cleaner protein profile in SDS-PAGE*
- *Pigment analysis through HPLC*
- *Resource allocation through FT-IR and elemental analysis*

Regulation of photosynthesis

In the model species *Chlamydomonas reinhardtii*, NPQ is regulated by the **LHCSR** pigment binding proteins. These can sense the acidification of the thylakoidal lumen that occurs in excess light and activate the dissipation of excess energy as heat. Yet, several other processes are regulating the photosynthetic electron transport and they might influence the heat dissipation mechanism.

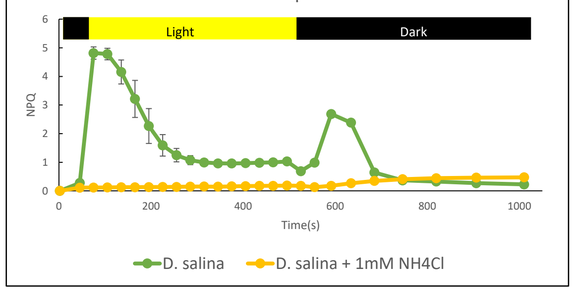


Possible photosynthesis regulatory elements: PTOX, Ndh-2, Violaxanthin de-epoxidase, LHCSR, FLV

To investigate what components of the photosynthetic apparatus may influence regulation of NPQ, we employed photosynthetic inhibitors.

NH_4Cl: Dissipates the proton gradient in the lumen.
The addition of NH_4Cl to the sample inhibits the activation of NPQ both in the light and in the dark conditions.

NPQ activation in the presence of NH_4Cl

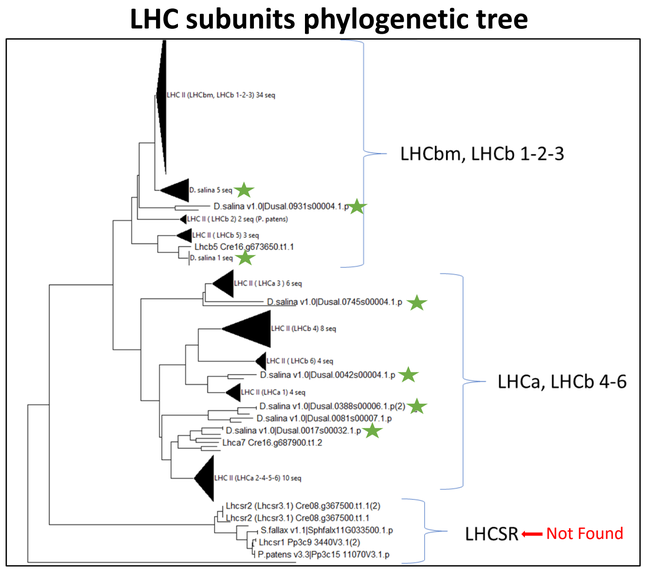


Other Inhibitors to be tested:

1. *P-gal: inhibits PTOX*
2. *DTT: inhibits Violaxanthin de-epoxidase*

Gene sequence research

LHC subunits phylogenetic tree



Most of the previous literature on *D. salina* genome focusses on genes related to salt adaptation, carbon metabolic pathways, glycerol and carotenoid production (Polle, et al., 2020).

We are searching the available genome for genes coding for proteins of the photosynthetic apparatus, e.g. LHC subunits, of which no sequence clusters with known LHCSR sequences.

This could be due to: missing regions of the genome OR different photosynthesis regulation?