Bridging the Gap between Short- and Long-Term Light Acclimation

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K_140351 (formerly: K_128950), Final Report

01.09.2018 – 31.12.2024

Photomorphogenesis of fresh water living cyanobacteria. Permanent excitonic decoupling of light-harvesting phycobilisomes from photosynthetic reaction centers in red-light grown *Cyanobium gracile*

Photomorphogenesis is a process by which photosynthetic organisms perceive external light parameters, including light intensity and quality (color), and adjust cellular metabolism, growth rates and other parameters, in order to survive in a changing light environment.

One of the main focus of the project was to comprehensively study long-term light (color) acclimation of selected cyanobacterial strains in term of pigment composition, light harvesting- and distribution, redox homeostasis, and cellular plasticity, and to explore the closely related interplay between short- and long-term light acclimation processes in these strains. The microorganism used in these investigations were the model cyanobacterium *Synechocystis* spp. 6803 (*S*. 6803), two *Cyanobium gracile* strains, ACT 63 (=ACT 1026), and ACT 9802, isolated from Hungarian lakes (Lake Fertő and Lake Balaton, respectively), but common also in other turbid shallow freshwater systems. Living in such optically complex environment makes *C. gracile* an ideal experimental object for studying light-acclimation of cyanobacteria in natural environments. On the other hand, exploring the most well-studied cyanobacterium, *S*. 6803, is also crucial for better understanding of these processes, as well as for creating a mathematical model to describe the light color dependency of various photosynthetic and cellular functions in cyanobacteria. Beside these, a phycoerythrin-rich cyanobacterium (ACT 9807), also isolated from Lake Balaton, and a *Nostoc* strain have also been intensively studied. These latter results are still under evaluation and not published yet. We grew the selected strains in semi-continuous batch cultures under monochromatic lights (435, 465, 495, 520, 560, 596, 615, 633, 663, and 687 nm; covering the whole visible spectrum), provided by narrow-band power LEDs.

We performed UV-Visible absorption spectroscopy, low-temperature (77 K) fluorescence emission- and excitation spectroscopy, and pigment analysis in order to determine antenna- and cellular pigment composition, as well as Photosystem II to Photosystem I (PSII to PSI) ratios in the selected strains under various light conditions. To quantify PSII and PSI-mediated and cyclic electron transport we applied saturating pulse methods using a Dual-PAM-100 (Dual-PAM) measuring system purchased *via* the project. Cellular localization of pigment-protein complexes (i.e., PS II and phycobilisomes, PBS´s) was determined by confocal microscopy. Capability of the cells for short-term light acclimation processes was probed by fluorescence induction curves and rapid light curves using Dual-PAM and a Multi-Color-PAM provided a partner organization.

Growth rates of both *C. gracile* and *S*. 6803 was very wavelength-dependent. Similarly to other cyanobacteria, they grew slowly under violet, blue, or green light, and relatively fast under red light. Quantitatively, the lowest and highest growth rates were measured at around 465–495 nm and from 596 nm to 663 nm, respectively. The observed slow growth at the blue-blue green spectral region was accompanied by low maximal culture densities. Reaching the far-red region (687 nm) involved considerably slower growth rates again. Growth rates correlated well with cell volumes, albeit the maximal relative differences in cell volumes were much smaller (~10%) than the maximal differences in growth rates (7-fold). Nevertheless, the insufficient microbial growth under sub-optimal light conditions was partially compensated by smaller cell volumes.

Not surprisingly (and much more importantly!), growth rates also correlated perfectly with PSII-mediated electron transport rates, ETR(II), which were the lowest, again, in the violet to green spectral region (at 465 nm and 495 nm in *C. gracile* and 435 nm to 520 nm in *S*. 6803), and was the highest from 596 nm to 663 nm. The respiratory and PSI-mediated electron transport rates, ETR(I), followed a similar pattern, however, with pretty low cyclic ET contribution under unfavorable light conditions. These, again, were partly compensated by higher efficiencies in short-term light acclimation processes (i.e. state transition and OCP-quenching) in cultures grown either under violet to green or under 687 nm light.

The minimal ET (and growth) rates under the violet to green light could not be explained by the absorbance profile of the cells as photosynthetically usable radiation (PUR, product of radiation and absorption spectra) was the highest at the violet to blue-green

region. The absorbance spectra of these strains were similar to those of other cyanobacteria with a major chlorophyll *a* (Chl *a*) Soret band at 440 nm and a Q-band at 680 nm, a carotenoid shoulder at 485 nm, and phycocyanobilin absorption peaked at 625 nm. The most dramatic changes in the absorbance spectra of both *C. gracile* and *S.* 6803 grown under various monochromatic lights were observed in the latter spectral region. Plotting the A625/A680 ratios against growth wavelengths revealed high PBS to Chl ratios over the violet to blue-green and in the near-infrared region with the highest A625/A680 ratios at around 465–495 nm and 687 nm, and a low, stable PBS abundance between these growth wavelengths. The remarkably high absorbance and also the modified spectral shape in the blue spectral region of 687 nm-grown *C. gracile* cells suggests an elevated carotenoid level in these cells, which is also supported by a more intense carotenoid shoulder at 485 nm.

This assumption, i.e. high carotenoid levels in cyanobacteria grown under near-infrared (and also under violet) light was confirmed by high performance liquid chromatography (HPLC) data. In each case, chromatograms were dominated by three major bands, originated from zeaxanthin, Chl *a*, and β-carotene, respectively. In good agreement with growth rates and cell sizes, total pigment content of the cultures were the lowest at the growth lights of 465–495 nm and, again, at 687 nm. Regarding pigment composition, zeaxanthin to Chl *a* ratio was significantly high in the cells grown under 687 nm, while the β-carotene content did not show any statistically significant change. A substantial increase in both carotenoid levels were observed in the cells grown at 435 nm; however, these increases were due to the decrease of Chl *a* content in these samples, rather than due to an increase in the carotenoid levels. Nevertheless, the increased (relative) carotenoid levels, indicate a dual light-harvesting/photoprotective role of carotenoids under critical light conditions.

Excitation energy transfer and PSII-to-PSI ratios were examined by low temperature (77 K) fluorescence emission spectroscopy. Spectra with 455 nm (Chl *a*) excitation showed three major spectral features: a minor phycobiliprotein emission at 655 nm; a double emission peak at 685–695 nm, originated from PSII with a variable contribution to the signal by the PBS terminal emitter (emitting at 685 nm only); and a robust PSI emission peaked at 728 nm, indicating small PSII/PSI ratios over the whole visible range, regardless of the growth wavelength. Nevertheless, increasing the growth wavelength from 435 nm to 663 nm results in a remarkably, about 2-fold increase and a 3-fold decrease in the PSII/PSI ratios

in *C. gracile* and *S.* 6803, respectively. The proportional changes of the 685 nm and 695 nm emission suggests only a minor contribution of the PBS terminal emitter. In case of *C. gracile*, cells grown under 687 nm light show an intense fluorescence emission at 685 nm and a distorted shape of the 685–695 nm double band, showing a considerable light emission from the PBS terminal emitter.

This feature was much better seen with direct PBS excitation at 590 nm. These spectra revealed that PBS decoupling, at a certain extent, takes place also at lower growth wavelengths but it becomes dominant at 687 nm. Enhancing fluorescence intensities of the 655 nm emission as compared to the PSI emission at 728 nm were in good accordance with increasing PSII-to-PSI ratios, and rather than showing increasing PBS levels, they indicated changes in the excitation energy transfer. Besides these, the spectra also showed the intensification of two shoulders, at 745 nm and 765 nm, respectively. By fluorescence excitation spectroscopy we identified their origin as PBS. We explored the unique fluorescence properties of *S.* 6803 and *C. gracile* cells grown under 687 nm light also by confocal microscopy. While confocal micrographs of the control cells showed typical cellular patterns with intense, ring-shaped autofluorescence from the cell periphery, where thylakoid membranes are located, cells grown under 687 nm light show a more homogenous pattern, dominated by PBS fluorescence in the cytoplasmic space.

In summary, according to incident light, cyanobacterial cells perform great plasticity in term of pigment composition, antenna size, and photosystem stoichiometry to optimize their photosynthetic performance and to redox poise their intersystem electron transport chain. Also, short-term like acclimation processes are typically more effective in cyanobacteria grown under unfavorable light conditions. In spite of such compensatory/survival strategies, *S.* 6803 and *C. gracile*, like other cyanobacteria, uses blue and near far-red light less efficiently, which involves moderate growth rates and reduced cell volumes. Increasing the wavelength of the growth light is accompanied by changing PSII to PSI ratios, while under unfavorable light conditions, where neither chlorophyll nor PBS´s absorb light sufficiently, are compensated either by an enhanced antenna size and/or carotenoid levels. In case of *C. gracile*, increasing PSII to PSI ratios, which involve better light utilization in the red spectral region, was accompanied by a partial excitonic antenna decoupling, which decoupling was the highest in the cells grown under 687 nm light. So far, similar phenomenon could have been induced only by strong light; we have demonstrated in

the project, that under certain physiological conditions it is also possible to induce such decoupling by weak light. This suggests that suboptimal photosynthetic performance of the near far-red light grown *C. gracile* cells is due to a solid redox- and/or signal-imbalance, which leads to the activation of this short-term light acclimation process.

Three manuscripts were prepared based on these findings. The data on *C. gracile* have already been published (Bernát et al. Front. Plant Sci. (2021) 12: 612302), while two *Synechocystis* manuscripts are under revision and just before re-submission. These manuscripts are deposited in repositories under doi's 10.1101/2023.06.08.544187v1 and 10.1101/2023.06.30.547186v1.

Essential part of the project was collecting strains from Hungarian water bodies. As a side result, high (in some cases: extremely high) abundance of anoxygenic autotrophic bacteria were found in Hungarian shallow lakes during collection of strains. This has been reported in Szabó-Tugyi N et al. (2019) FEMS Microbiology Ecology 95: fiz104.

Environmental acclimation of marine cyanobacteria

We extended the above described work with studying the environmental (including light) acclimation of marine diazotrophic (*Crocosphaera watsonii*, *Cyanothec*e sp. ATCC 51142) cyanobacterial strains growing either under N-limited and N-replete conditions. We concluded that in-depth understanding of nutrient cycling/budget under changing environmental conditions is very important for understanding of various (including light) acclimation processes. For that reason we designed a kinetic model to describe the photosynthetic performance of these model cyanobacteria. We reported our findings in the following papers: Masuda et al. (2023) Comput. Struct. Biotech. J. 21: 58-65; Inomura et al. (2021) Comput. Struct. Biotech. J. 19: 6456-6464; Erratum: Comput. Struct. Biotech. J. 20: 385; Rabouille et al. (2021) Front. Microbiol. 12: 617802; Polerecky et al. (2021) Front. Microbiol. 12: 620915.

Light color acclimation of eustigmatophyte algae

We performed UV-Visible absorption spectroscopy, low-temperature (77 K) fluorescence emission spectroscopy, and pigment analysis in order to determine antenna- and cellular pigment composition, as well as PSII to PSI ratios of the eustigmatophyte algae *Vischeria*

stellata and the isolate FP5 under various light conditions. To quantify PSII and PSI-mediated as well as cyclic electron transport we applied saturating pulse methods using a Dual-PAM-100 (Dual-PAM) measuring system. Cellular localization of pigment-protein complexes (i.e., PSII, PSI, far-red (FR)-antenna) was determined by confocal microscopy. Capabilities of the cells for short-term light acclimation processes was probed by recording fluorescence induction curves and rapid light curves (with both red and blue actinic light) by Dual-PAM-100. Based on preliminary results, we paid special attention to cultures grown under 690 and 705 nm, and also under blue light. We found that acclimation to FR-light is quite beneficial in term of survival in FR-enriched niches; however these cells are quite vulnerable to visible light. Altered PQ-diffusion in a reorganized membrane architecture is possibly a key issue here and presence of blue light (and blue-light sensing photoreceptors) seems to be essential for a rapid light response. One manuscripts is under preparation based on these findings.

Environmental acclimation of benthic and soil living algae

Beside the above described cyanobacteria and eustigmatophyte algae we also examined the environmental (including light) acclimation of benthic and soil living algae. The former was performed using naturally formed biofilms built on solid surfaces, while the latter was carried out using a cave-isolated filamentous green alga (*Klebsormidium* sp.). Regarding *Klebsormidium* sp., we determined the temperature and light optimum of the photosynthetic performance and growth of this strain with potential biotechnological applications due to its high brassinosteroid content. We reported these findings in Lengyel et al. (2023) Environ. Res. 238: 117283 and Futó et al. (2024) J. Appl. Phycol. *ONLINE FIRST* doi: 10.1007/s10811-023-03161-2.

Other results

As a side result of the project, two review/analysis papers and two methodological papers were also published: Bernát et al. (2020) Hydrobiologia 847: 3999–4013; Somogyi et. al. (2020) Biol. Fut. 71: 371–382; Greipel et al. (2024) Biol. Fut. *ACCEPTED*; Futó et. al (2024) Acta Physiol. Plant. *ACCEPTE*D