



### ADEVERINȚĂ

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## Effect of microaerobiosis on photosystem II in *Synechococcus* sp. PCC 7002

Iuliana Chiş<sup>1,2</sup>, Bogdan Druga<sup>3,4</sup>, Dalton Carmel<sup>1,5</sup>, Ciprian Chiş<sup>1,2</sup>, Aurel Ardelean<sup>5</sup>, Nicolae Dragoş<sup>2</sup>, Cosmin Ionel Sicora<sup>1,✉</sup>

**SUMMARY.** Cyanobacterial oxygenic photosynthesis had transformed the early earth's biosphere and resulted in complex aerobic life forms. During the course of evolution, cyanobacteria retained many genes that respond to specific environmental cues. Our study shows that microaerobic treatment did not significantly alter the functionality of photosystem II complex in *Synechococcus* sp. PCC 7002 strain. Surprisingly no changes in the induction levels of *psbA* genes, especially in D1' isoform, had been recorded. This finding is important as signals an atypical behaviour of D1' isoform from *Synechococcus* sp. PCC 7002 to microaerobic stress as compared to other cyanobacterial strains.

**Keywords:** Chlorophyll, Cyanobacterium, Fluorescence, Microoxic, *psbA*, RT-PCR

**Abbreviations:** PSI- photosystem I, PSII- photosystem II, Q<sub>A</sub>- first quinone acceptor in PSII, Q<sub>B</sub>- second quinone acceptor in PSII, DCMU- (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), qRT-PCR- quantitative reverse transcriptase polymerase chain reaction, O<sub>2</sub>- oxygen, UV-B- ultraviolet radiation B

### Introduction

Cyanobacteria experiences low oxygen concentration under several habitats such as hot springs and in specially modified cells, where photosynthetic rate is

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<sup>1</sup> Biological Research Centre, Jibou, Romania

<sup>2</sup> Babeş-Bolyai University, Cluj-Napoca, Romania

<sup>3</sup> Institute of Biological Research, Department of Taxonomy and Ecology, Cluj-Napoca

<sup>4</sup> Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland

<sup>5</sup> Western University "Vasile Goldis", Arad, Romania

✉ **Corresponding author: Cosmin Sicora, Biological Research Centre, Jibou, Romania,**

E-mail: [cosmin.sicora@gmail.com](mailto:cosmin.sicora@gmail.com)

limited (Voorhies et al., 2012). Their adaptation to these anaerobic environments and their transition to aerobic conditions and *vice versa* remains important in understanding the regulation of photosynthesis (Summerfield et al., 2011). On the scale of complexity of photosynthesis, contemporary cyanobacteria, resembles closely to higher plants (Mulikidjanian et al., 2006). The cyanobacterial oxygenic photosynthesis is a multiplex cumulative process performed by diverse pigment-protein complex located in thylakoid membranes inside cyanobacteria (Blankenship 2014). The vital protein complex include photosystem II (PSII), NADH-quinone oxidoreductase complex-1, Cytochrome  $b_6f$  and photosystem I (PSI) (Chis et al., 2014). The PSII plays crucial role of catalyzing the splitting of water and leads to the generation of 4 electrons (Mulo et al., 2009). The components at PSII donor side shuttles these electron to the acceptor side via different cofactors, many of them being coordinated by the core D1 protein, which is coded by the gene *psbA* (Rast et al., 2015).

Cyanobacteria variably contain 1–5 copies of *psbA* coding for 1–3 unique D1 isoforms per species (Wegener et al., 2015). The D1 isoforms are grouped as D1m, D1', D1:1, and D1:2 (Sicora et al., 2006). D1m is a protein isoform that is regularly expressed under normal growth conditions and is induced under stressful conditions such as various environmental cues (Sane et al., 2002). The D1:1 is expressed during normal growth conditions but repressed under stress, where as in contrast, D1:2 is induced to replaces D1:1 in PSII reaction centers upon exposure to unusual growth conditions such as high light, cold temperature, UV-B radiations etc. (Sicora et al., 2006, Sicora et al., 2008, Vinyard et al., 2013). The D1' is always induced under low O<sub>2</sub> or microaerobic conditions. The previously believed silent *psbA* genes in cyanobacteria, encoding D1' isoform is a distinct functional group with a unique regulation mechanism responding to specific cellular needs. It is well distinguished from other PsbA isoforms by the consensus amino acid replacements at position 80 (Gly to Ala), 158 (Phe to Leu) and 286 (Thr to Ala) (Sicora et al., 2009).

*Synechococcus* sp. strain PCC 7002 genome has three *psbA* genes, *a1418* (PsbA1), *a0157* (PsbA2) and *a2164* (PsbA3), which encode three D1 isoforms. These natural variants of D1 subunits tune photochemical PSII fitness to varying solar radiations (Vinyard et al., 2013). The protein sequence of the PsbA1 and PsbA2 isoforms exhibits 100% identical amino acids. *a2164* encodes a putative D1' form which exhibits these three key changes in the amino acid compositions at the respective positions (Mulo et al., 2009). Although the induction of D1' isoform, has been studied in some cyanobacterial strains, their functional role towards photosynthesis remains widely unknown. In our study we aim to assess the expression pattern of the various *psbA* genes and on the overall functionality of PSII complex, under microaerobic conditions in *Synechococcus* sp. PCC 7002 a cyanobacterium strain of interest in recent years.

## Materials and methods

### *Strain, growth and treatment conditions*

The wild type *Synechococcus* sp. strain PCC 7002 was grown in flasks with medium A<sup>+</sup> containing 1 mg NaNO<sub>3</sub> ml<sup>-1</sup> at 38°C (Stevens et al. 1973). Light was provided by cool-white fluorescent lamps (250 µmol m<sup>-2</sup> s<sup>-1</sup>). The photon flux density was measured using a QSPAR Quantum Sensor (Hansatech Instruments Ltd, Norfolk, United Kingdom) light meter while cell growth was monitored by the optical density at 550 nm (OD<sub>550</sub>) with a model Shimadzu UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Microaerobiosis was achieved by argon bubbling in the culture flasks for up to 60 minutes. After 10 minutes the oxygen concentration was found to be below 5% compared to the original value, as measured with an oxygen sensor (Mettler Toledo AG, Switzerland). The culture was then returned to aerobic conditions following bubbling with air for 60 min. Subsequent to stress treatments the cells were returned to normal conditions (60 minutes recovery). 12 ml aliquots were sampled after 0, 15, 30, and 60 min of stress conditions, as well as after 30 and 60 min of recovery period for Real-Time Quantitative PCR (qPCR) measurements.

### *Flash-induced fluorescence measurements*

Flash-induced intensification and subsequent decline of fluorescence was assessed using a double-modulation fluorometer (PSI Instruments, Brno, CZ). Both measuring (2.5 µs) and actinic (20 µs) flashes were produced by red LEDs. All the measurements were completed in the interval of 150 µs to 100 s, the measuring flashes being applied in a logarithmic series and in the presence of the Photosystem II inhibitor 3-(3', 4'-dichlorophenyl)-1,1-dimethylurea (DCMU) at a final concentration of 10 µM, in order to block the transfer of electrons between Q<sub>A</sub> and Q<sub>B</sub>. To minimize the distortion of the relaxation kinetics due to the actinic effect, the intensity of the measuring flashes was adjusted to a low value. *Synechococcus* sp. PCC 7002 cells at 4 µg chl ml<sup>-1</sup> were adapted to dark for 10 min prior measurements. Analysis of the fluorescence decrease was based on the model of the two electron gate as described earlier (Vass et al., 1999).

### *Quantitative reverse transcriptase polymerase chain reaction*

Total RNA was extracted from cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and dissolved in nuclease free water (Thermo Scientific, Waltham, MA, USA). Each sample was treated with 1U DNA-ase (Ambion Turbo DN-ase, Austin, TX, USA) to avoid genomic DNA contamination. The concentration of the RNA solution was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The synthesis of first strand cDNA from 1 µg of purified RNA was completed with the First Strand

cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) using the random hexamer primers.

Two specific primer pairs were designed to amplify transcripts from the *psbA* genes in *Synechococcus* sp. PCC 7002. One of these primer pairs (Fwd 5'-TTGCAGCCCACGGCTACTTC 3' and Rev- 5'-GTTCAGAATGTCCGCCAGGT-3') amplifies a 221 bp fragment of the genes encoding the D1 protein (SYNPCC 7002\_A1418 and SYNPCC 7002\_A0157), while the other one (Fwd- 5'- CCACACTGTTGACCGCAACGAT-3' and Rev- 5'-GTAGGGGCCA CCGTTGTAGAG -3') targets a 220 bp fragment belonging to the *psbA* gene responsible for the synthesis of the D1' protein (SYNPCC7002\_A2164). Another primer pair (Fwd- 5'-GCTTATCGCTGCACTGGAGT-3' and Rev- 5'-GGCCGTTCTACTTTATTTCC-3') was designed for the *pepC* gene encoding phosphoenolpyruvate carboxylase, to be used as a reference gene.

## Results

The cells were subjected to microaerobic condition for 60 min by bubbling argon gas into the culture medium. After the treatment, the culture was returned to aerobic conditions following bubbling with air for 60 min. It is noteworthy to mention that the induction of microaerobic environment inside the culture itself is achieved by various methods including bubbling gases (such as N<sub>2</sub>, argon etc.) or by enzymatic reactions. In our study, we used real-time qRT-PCR technique to monitor the expression levels of the two D1 protein isoforms encoded by *psbA* genes from *Synechococcus* sp. PCC 7002 under growth conditions, microaerobic treatment and during recovery. Our results showed significant difference between the relative amounts of *psbA* transcripts. The D1 isoform remained dominant throughout the treatment and recovery contributing to 99.9% of the total *psbA* transcripts (Table 1). In general, microaerobic treatment did not greatly alter the expression of any of the *psbA* isoform expression in our study yet minor induction at 15 min followed by slight down regulation at 60 min was noticed (Fig. 1). The changes are negligible but significant and close to error threshold of the method. The unaltered expression of *a2164* gene during microaerobic treatment remains very unique in our study.

The functional characteristics of photosystem II were investigated using a double modulation fluorometer as described previously (Trtilek et al., 1997). We measured the flash-induced rise and subsequent decay of the fluorescence indicative of Q<sub>A</sub><sup>-</sup> reoxidation. This gives information on the number of active PSII centers at the time of the flash as well as the efficiency of electron transfer within the acceptor side of PSII. The presence of DCMU, by synchronizing the PSII centers in a blocked state, makes the amplitude of flash fluorescence a good estimation of the potential number of active centers while in the absence of DCMU

we see only the number of centers active at the time of the flash. During the 60 min of microaerobic treatment and the subsequent 60 min of recovery in normal air, at constant temperature and light intensity, we did not record a significant decrease in the number of active PSII centers both in the absence (Fig. 2a) or presence of DCMU (Fig. 2b). The microaerobic treatment did not significantly change the function of the PSII on the acceptor side of PSII (Fig. 2c) or donor side (Fig. 2d), an observation in accordance with previous studies done on different cyanobacterial species (Sicora et al., 2009).

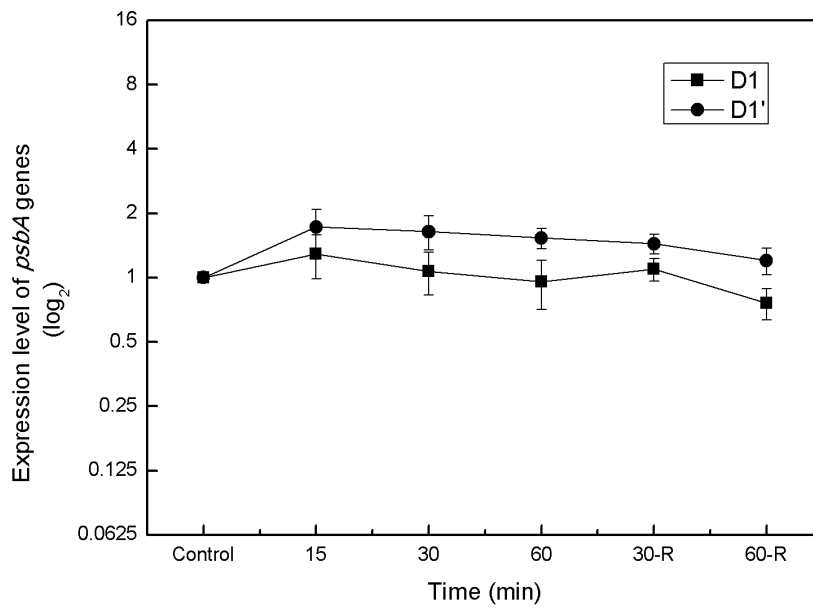
### Discussion

Previous studies in *Synechocystis* sp. PCC 6803 had shown that D1' isoforms are induced when the cells are subjected to microaerobic conditions (Sicora et al., 2009, Summerfield et al., 2008). Sequence comparison performed on three cyanobacterial strains where microaerobic induction of D1' was recorded showed the existence of three very specific amino acid changes that are always present in D1' protein forms (Sicora et al., 2009). *In silico* analysis performed on different *PsbA* sequence clearly showed *a2164* gene from *Synechococcus* sp. PCC 7002 showed all characteristic changes in the amino acids specific to D1' isoform (Mulo et al., 2009). The microaerobic conditions, achieved by bubbling argon gas to *Synechococcus* sp. PCC 7002 cells while maintaining all other growing parameters constant did not significantly change the function of the donor or acceptor side of photosystem II complex nor did it change the number of active PSII centers. This shows that for short periods of time (60 min) microaerobic conditions did not act as a stress factor to the photosynthetic apparatus in this cyanobacterial strain. In addition, the microaerobic treatment did not influence the induction of *psbA* gene family expression. The novel finding in our study is that the D1' like isoform, which responds to microaerobic conditions in other cyanobacteria, was not induced in *Synechococcus* sp. PCC 7002. It is for the first time that a member gene of the *psbA* gene family, encoding a protein isoform with the three typical amino acid mutations present, position 80 (Gly to Ala), 158 (Phe to Leu) and 286 (Thr to Ala), is not induced by microaerobic treatment.

**Table 1.**

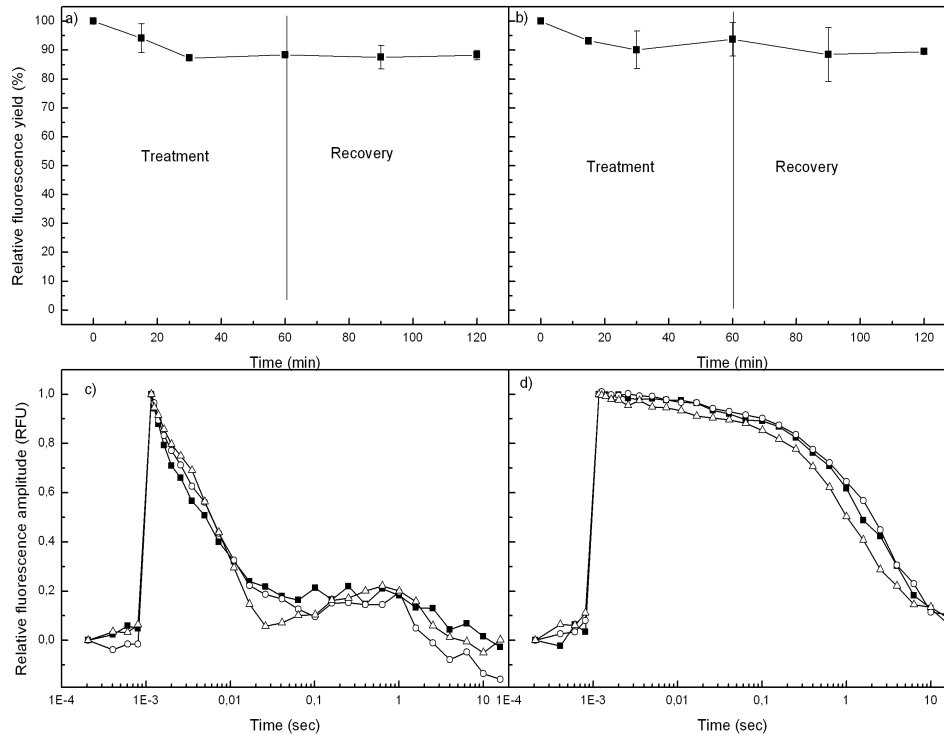
Relative transcript abundance of *psbA* isoforms under microaerobic treatment and during the recovery

| <i>psbA</i><br>isoforms<br>transcript | Microaerobic Treatment |         |         |         | Recovery |         |
|---------------------------------------|------------------------|---------|---------|---------|----------|---------|
|                                       | Control                | 15 min  | 30 min  | 60 min  | 30 min   | 60 min  |
| D1 (%)                                | 99.9759                | 99.9683 | 99.9552 | 99.9524 | 99.9612  | 99.9564 |
| D1' (%)                               | 0.0241                 | 0.0317  | 0.0448  | 0.0476  | 0.0388   | 0.0436  |



**Figure 1. Real Time quantitative reverse transcriptase PCR analysis:** qRT-PCR expression analysis of *psbA*-D1' (closed circle) and other D1 isoforms (closed square) under microaerobic stress (15 min, 30 min, 60 min) followed by recovery under growth conditions [90 min (30-R) and 120 min (60-R)] in *Synechococcus* sp. PCC 7002 strain.

EFFECT OF MICROAEROBIOSIS ON PHOTOSYSTEM II IN *SYNECHOCOCCUS* sp. PCC 7002



**Figure 2. Influence of microaerobic conditions on the PSII function in *Synechococcus* sp. PCC 7002.** Changes in the number of actual active centres (panel a) and potential active centres (panel b) at control, microaerobic treatment (15 min, 30 min, 60 min) and subsequent recovery under growth conditions (90 min and 120 min) in the absence (panel a) and presence of DCMU (panel b). The decay of flash-induced fluorescence was followed using measuring flashes on a logarithmic time scale in the absence (panel c) and presence of DCMU (panel d) at control (solid black squares), 60 min microaerobic treatment (open circles) and after 60 min of subsequent recovery (open up triangles) Changes in the shape of the curves were made evident by normalization of the decay curves to 1, during the treatment.

**Conclusions.** Our results show that *Synechococcus* sp. PCC 7002 do not increase the expression of the predicted D1' protein and there are no significant changes in the electron transfer pathways within PSII. It is for the first time such a response is recorded and we can conclude that *Synechococcus* sp. PCC 7002 contains an atypical, possibly entirely new form of D1 protein with, so far, an unknown function.

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EFFECT OF MICROAEROBIOSIS ON PHOTOSYSTEM II IN *SYNECHOCOCCUS* sp. PCC 7002

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