

=== SHORT COMMUNICATION ===

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

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SUMMARY. Cyanobacterial oxygenic photosynthesis transformed the early Earth's biosphere and resulted in complex aerobic life forms. During the course of evolution, cyanobacteria retained many genes that responded to specific environmental cues. Our study shows that microaerobic treatment did not significantly alter the functionality of photosystem II complex in *Synechococcus* sp. strain PCC 7002. Surprisingly, no changes in the induction levels of *psbA* genes, especially in D1' isoform, were 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_A- first quinone acceptor in PSII, Q_B - second quinone acceptor in PSII, DCMU - (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), qRT-PCR - quantitative reverse transcriptase polymerase chain reaction, O₂ - oxygen, UV-B - ultraviolet radiation B

Introduction

Cyanobacteria evolve low oxygen concentration under several habitats such as hot springs and in specially modified cells, where photosynthetic rate is limited (Voorhies *et al.*, 2012). Their adaptation to these anaerobic environments and their transition to aerobic conditions and *vice versa* remains important in understanding

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the regulation of photosynthesis (Summerfield *et al.*, 2011). On the scale of complexity of photosynthesis, contemporary cyanobacteria resemble closely to higher plants (Mulkidjanian *et al.*, 2006). The cyanobacterial oxygenic photosynthesis is a multiplex cumulative process performed by diverse pigment-protein complexes located in thylakoid membranes inside cyanobacteria (Blankenship, 2014). These vital protein complexes include photosystem II (PSII), NADH-quinone oxidoreductase complex-1, Cytochrome b_6f and photosystem I (PSI) (Pakrasi *et al.*, 1985; 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 electrons to the acceptor side via different cofactors, many of them being coordinated by the core D1 protein, which is coded by the *psbA* gene (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 regularly expressed under normal growth conditions and 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_2 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 contains three *psbA* genes, *a1418* (PsbA1), *a0157* (PsbA2), and *a2164* (PsbA3), encoding for 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% amino acids similarity. *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 levels of the various *psbA* genes and on the overall functionality of PSII complex, under microaerobic conditions in *Synechococcus* sp. PCC 7002.

Materials and methods

Strain, growth and treatment conditions

The wild type *Synechococcus* sp. strain PCC 7002 was grown in flasks with medium A⁺ containing 1 mg $NaNO_3$ ml^{-1} at 38°C (Stevens *et al.* 1973). Light was provided by cool-white fluorescent lamps (250 μmol m^{-2} s^{-1}). The photon flux density

was measured using a QSPAR Quantum Sensor (Hansatech Instruments Ltd, Norfolk, UK) light meter while cell growth was monitored by the optical density at 550 nm (OD₅₅₀) with a 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 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, Czech Republic). Both measuring (2.5 μ s) and actinic (20 μ s) flashes were produced by red LEDs. All the measurements were completed in the interval of 150 μ s to 100 s, the measuring flashes being applied in a logarithmic series and in the presence of the PSII inhibitor 3-(3', 4'-dichlorophenyl)-1,1-dimethylurea (DCMU) at a final concentration of 10 μ M, in order to block the transfer of electrons between Q_A and Q_B. 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 μ g chl ml⁻¹ 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 μ g 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 (F 5'-TTGCAGCCCACGGCTACTTC 3' and R 5'- GTTCAGAAATGTCCGCCAGGT-3') amplifies a 221 bp fragment of the genes encoding the D1 protein (SYNPCC 7002_A1418 and SYNPCC 7002_A0157), while the other one (Forward- 5'-CCACACTGTTGACCGCAACGAT-3' and Reverse- 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'-GGCCGCTTCTACTTTATTTTCC-3') was designed for the *pepC* gene encoding phosphoenolpyruvate carboxylase, to be used as a reference gene as its expression has been previously proven constitutive under conditions similar to our (Sicora *et al.*, 2006).

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 N₂, 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, and also chlorophyll fluorescence measurements to highlight the functional characteristics of the donor and acceptor side of photosystem II during control, the microaerobic treatment and recovery.

qRT-PCR results

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, in our case around the value of 2 fold. The unaltered expression of *a2164* gene during microaerobic treatment remains very unique in our study.

Table 1.

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

<i>psbA</i> isoforms 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

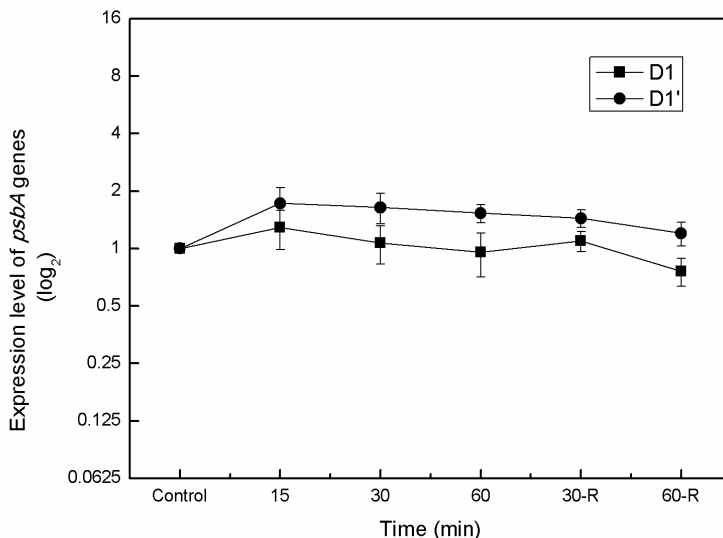


Figure 1. Real Time quantitative reverse transcriptase PCR analysis: qRT-PCR expression analysis of *psbA*-D1' (closed circle) and other D1 isoforms (closed square) under microaerobic stress (15 min, 30 min, 60 min) followed by recovery under growth conditions [90 min (30-R) and 120 min (60-R)] in *Synechococcus*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

Chlorophyll fluorescence measurements results

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_A^- 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 performed on different cyanobacterial species (Sicora *et al.*, 2009).

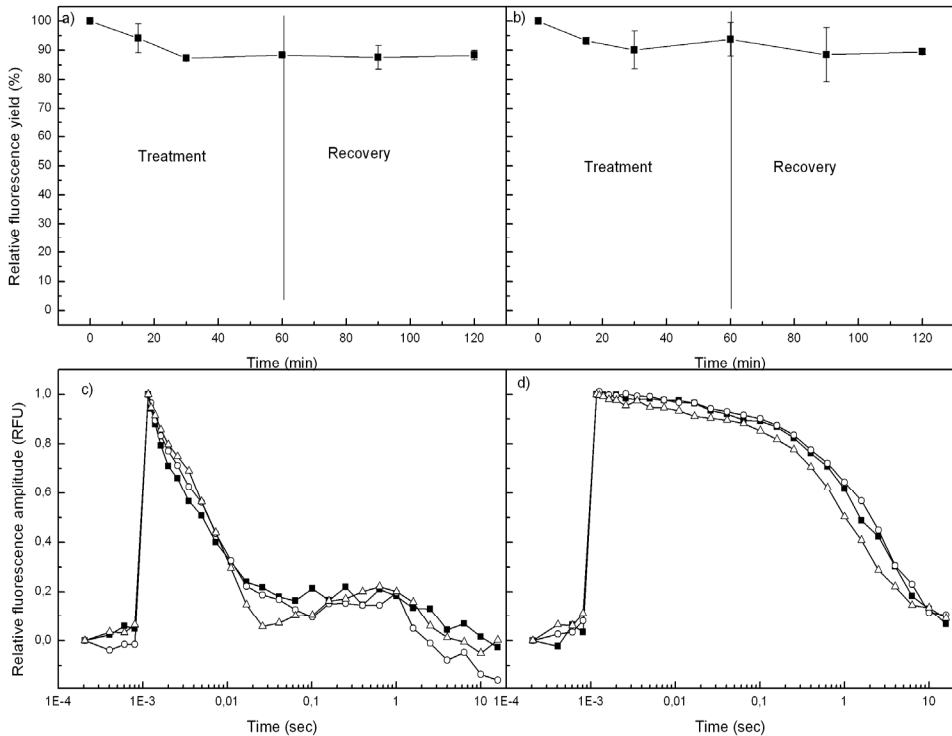


Figure 2. Influence of microaerobic conditions on the PSII function in *Synechococcus* sp. PCC 7002. Changes in the number of actual active centres (panel a) and potential active centres (panel b) at control, microaerobic treatment (15 min, 30 min, 60 min) and subsequent recovery under growth conditions (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.

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 microaerobical 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 protein sequence clearly showed that *a2164* gene from *Synechococcus* sp. PCC 7002 has

all characteristic changes in the amino acids specific to a D1' isoform (Mulo *et al.*, 2009). Both function of the donor or acceptor side of photosystem II complex and the amount of active PSII centers did not significantly change in *Synechococcus* sp. PCC 7002 tested under microaerobic conditions. These findings suggest 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 unprecedented 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 short-term microaerobic treatment.

Further studies will be needed to confirm this protein as a D1' isoform or to establish it as a different and yet unknown class of D1 protein.

Conclusions

Our results indicated 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 that such response is recorded and allow us further speculate 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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