Scientific report

*regarding the implementation of the project ''**The functional diversity of D1 proteins in photosystem II in cyanobacteria**'' code PN-II-ID-PCE-2011-3-0765 during January-December 2013*

The D1 protein of photosystem II (PSII), encoded by the *psbA* genes, is an indispensable component of oxygenic photosynthesis. Due to the strongly oxidative chemistry of PSII, D1 protein is subjected to constant deterioration, its replacement by a new copy being necessary every 5h in weak light conditions and every 20 minutes in intense light conditions, while most of the other components in PSII remain undamaged. In cyanobacteria, D1 protein is codified by the *psbA* gene family, which contains between 1 and 6 members. The presence of multiple genes which codify different isoforms of D1, is a clear indicator of their importance in the regulating mechanisms responsible for maintaining a functional PSII during the changing of environmental conditions in cyanobacteria natural habitats.

After the initial implementing phase of the project, in 2011, when the preliminary activities were performed, and in 2012, when the studies for the functional characterization of D1 protein were started, in 2013 we:

- Continued the studies regarding the functional characterization of D1 protein forms found in the model cyanobacteria cultures, which have a fully sequenced genome.
- Initiated the studies regarding the functional characterization of the D1 protein forms from cyanobacteria communities. The purpose of these studies is to establish a correlation between the different isoforms of D1 protein in specific environmental conditions.
- Optimized the protocols and working methods for the study of the function of D1 protein outdoor, during both day and night.
- Collected a large volume of experimental data through these experiments, which we present in several scientific articles. A synthesis of these data is also presented in this report.

The species considered in this phase of the study are: *Synechocystis sp.* PCC6803; *Cyanothece* ATCC51142 and samples taken from different cyanobacteria communities, for example the cyanobacteria communities from the hot spring in Ciocaia, Bihor district.

1. The influence of CO_2 on the expression of *psbA* gene from *Synechocystis sp.* PCC 6803

In *Synechocystis sp.* PCC6803 there are three *psbA* genes with different regulation strategies. The *psbA2* gene is responsible for expressing D1 protein in normal growth conditions, *psbA3* was described as the gene that responds at UVB treatments, while *psbA1*, initially considered a silent gene, proved to be induced in low oxygen or microaerobic conditions.

The purpose of the experiments was to investigate the manner in which *psbA1* gene is influenced by the CO_2 concentration in the environment, information which could help us understand how this gene is regulated in the wide context of cell adapting to environmental conditions.

Synechocystis sp. PCC6803 was obtained from Pasteur Culture Collection and grown at 30ºC and 50µmol photons $m^{-2}s^{-1}$ illumination. The strain was grown up to a chlorophyll concentration of 6µg chl ml^{-1}.

In order to investigate the influence of CO_2 on the *psbA* genes expression, we developed 3 series of experiments:

- In the first experiments, a mixture of 5% CO_2 95% air was continuously added to the control sample, next, air was continuously added to the culture for 120 minutes and then the initial gas mixture was added for another 60 minutes of recovery.
- In the second series of experiments, the air was replaced by synthetic air, without CO_2, for 48 hours, with no recovery period.
- In the third series of experiments, the cultures were grown by adding air, then the air was replaced with N_2 for 120 minutes and last the air was added for a recovery period of 60 minutes.

RNA and fluorescence samples were taken from all 3 cultures. The fluorescence measurements were made using a FL3500 fluorometer from Photon Systems Instruments, using a Q_A recombination protocol, and then the results were processed in Origin.8 software. A Joliot correction was applied to each sample

before doing the graphic. The *psbA* genes expression from the RNA samples was analyzed using a quantitative RT-PCR-IQ5 system, from Bio-Rad.

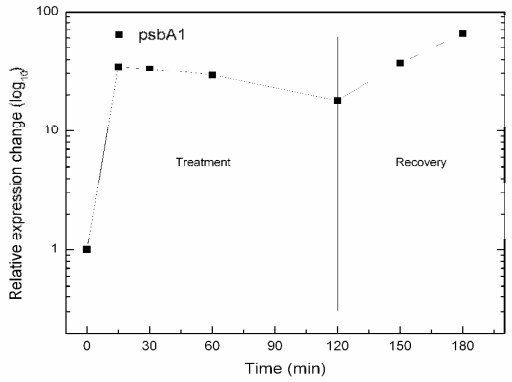


Figure 1. The effect of atmospheric air on the expression of *psbA1* gene in *Synechocystis sp.* PCC 6803.

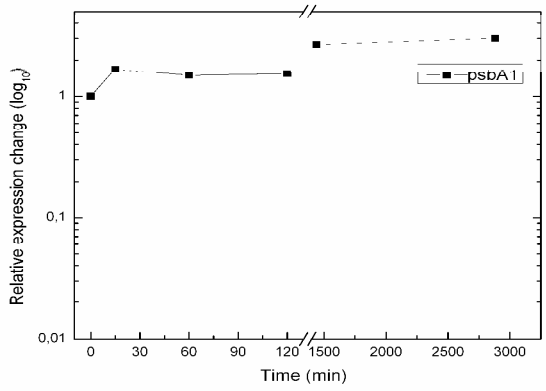


Figure 2. The effect of synthetic air on the expression of *psbA1* gene in *Synechocystis sp.* PCC 6803.

In figure 1 it can be observed, for the first time, an induction of the *psbA1* gene in the presence of atmospheric air, while switching the culture from air to synthetic air, shows a weak induction of the *psbA1* gene (Figure 2).

The third experiment, when N_2 is added in the culture, was used as a control experiment, in order to prove the expression of *psbA1* gene in microaerobic conditions.

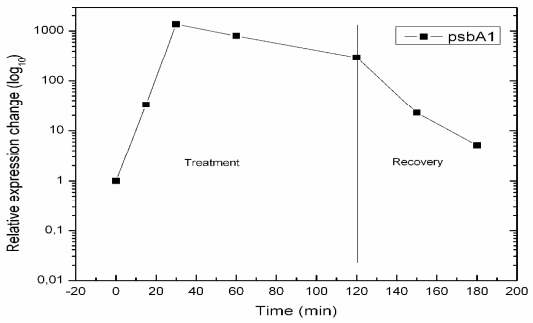
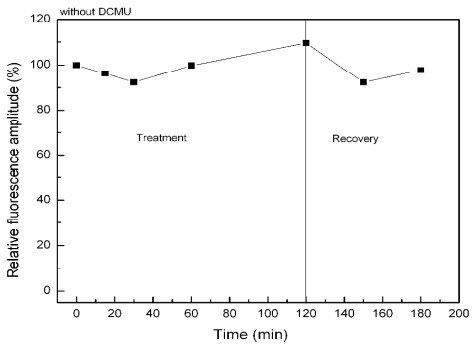


Figure 3. The effect of N_2 on the expression of *psbA1* gene in *Synechocystis sp.* PCC 6803.

a)



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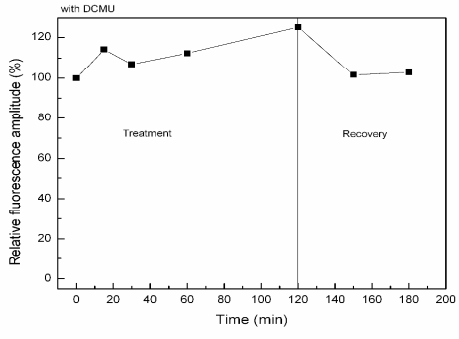

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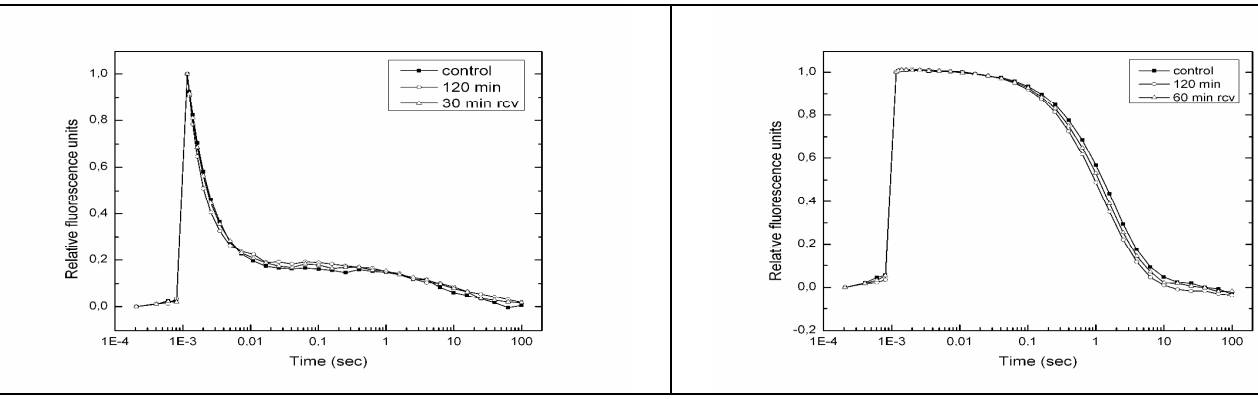


Figure 4. The effect of microaerobic conditions on the fluorescence amplitude in both the absence of DCMU (panel a) and presence of DCMU (panel b). The effects of the donor part (panel c) and the acceptor part (panel d) of the electron transporting chain in PSII are also presented.

The reduction of atmospheric O_2, due to the addition of N_2, had no notable effect on the number of active complexes in PSII, as can be observed in figure 4, in the absence (panel a) or presence (panel b) of DCMU.

In the absence of DCMU (panel c), the addition of N_2 causes an acceleration of the rapid phase of the electron transport chain, which suggests an alteration of the transfer between Q_A and Q_B.

In the presence of DCMU (panel d), an effect of the microaerobic conditions on the donor part of PSII can be observed, highlighted by an acceleration in the middle phase on the reoxidation of Q_A^- curve.

In conclusion, through these experiments we can observe an induction of *psbA1* gene when the CO_2 concentration is modified from 5% to air level while the O_2 concentration remains the same, induction also observed in the case of total elimination of CO_2 from air, in the presence of O_2. As previously described, the addition of N_2, which eliminates both CO_2 and O_2, has an inductive effect on the *psbA1* gene.

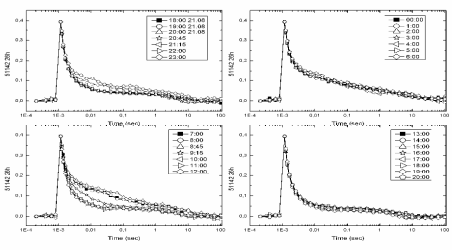
2. The influence of circadian rhythm on the function of PSII in day-night-cycle conditions at some model cyanobacteria species

In 2012, James Murray published an article called ‘‘Sequence variation at the oxygen-evolving center of photosystem II: a new class of ‘‘rogue’’ cyanobacteria D1 proteins’’, in which, following some bioinformatics studies, he documents a new isoform of D1 protein, the D1 rogue (D1r), induced in dark cycles, as well as specific locus in the protein amino acid sequence where modifications occur, for the case of D1r protein.

In order to verify how cyanobacteria cultures respond to day-night cycle or light-dark, we developed experiments for two model cyanobacteria strains, *Synechocystis sp.* PCC6803 and *Cyanothece* ATCC51142. The *Cyanothece* ATCC 51142 species has 5 *psbA* genes which codify different isoforms of D1 protein.

The experiments were realized in a light-dark 12h-12h cycle and RNA, protein, chlorophyll fluorescence samples were taken, for 26 hours. The results were processed in Origin.8. Both cyanobacteria cultures were brought to a chlorophyll concentration of 6μg chl ml^{-1}, in order to examine the effect of day-night cycle on the electron transport chain, but also on *psbA* genes and their expression, as well as on the quantity of D1 protein. The climatic chamber was set to turn the light off at 21:00 and turn it on at 09:00 o’clock.

a)



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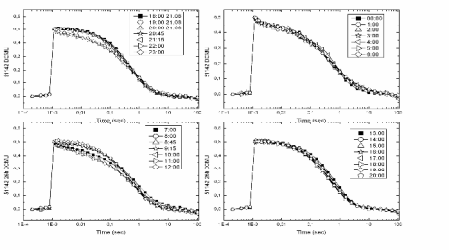

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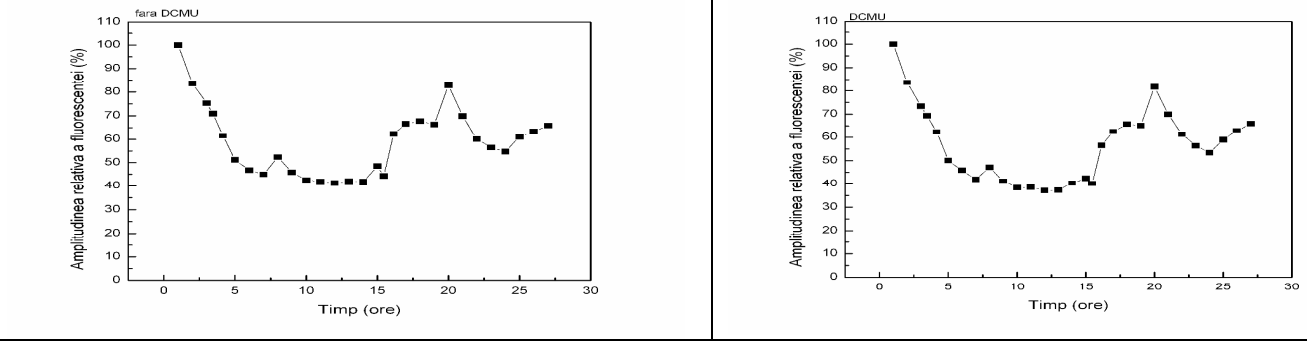

Figure 5. The effect of the circadian rhythm on the electron transport chain of PSII, both on the acceptor part of PSII (panel a) and on the donor part (panel b). The effect of day-night cycle on the relative amplitude of fluorescence in the absence (panel c) and presence (panel d) of DCMU.

It can be observed an acceleration of the middle phase in the acceptor part of the electron transport chain at 1h after the dark cycle started, effect which is present during the whole dark period (panel a) and it's correlated with a decrease of approximately 60% in the number of active centers of PSII, followed by a comeback of 30% during day cycle. In the donor part of the electron transport chain, a rapid phase can be observed in the presence of DCMU (panel c), which appears at the same time with the dark period and represents the recombination of Q_A with the cofactors close to the donor part, being caused by an inhibition of the water oxidation complex. The modifications which appear in the donor part of the electron transport chain are also correlated with a decrease in the number of active centers of PSII of approximately 60%, followed by a comeback of 30% during day period (panel d).

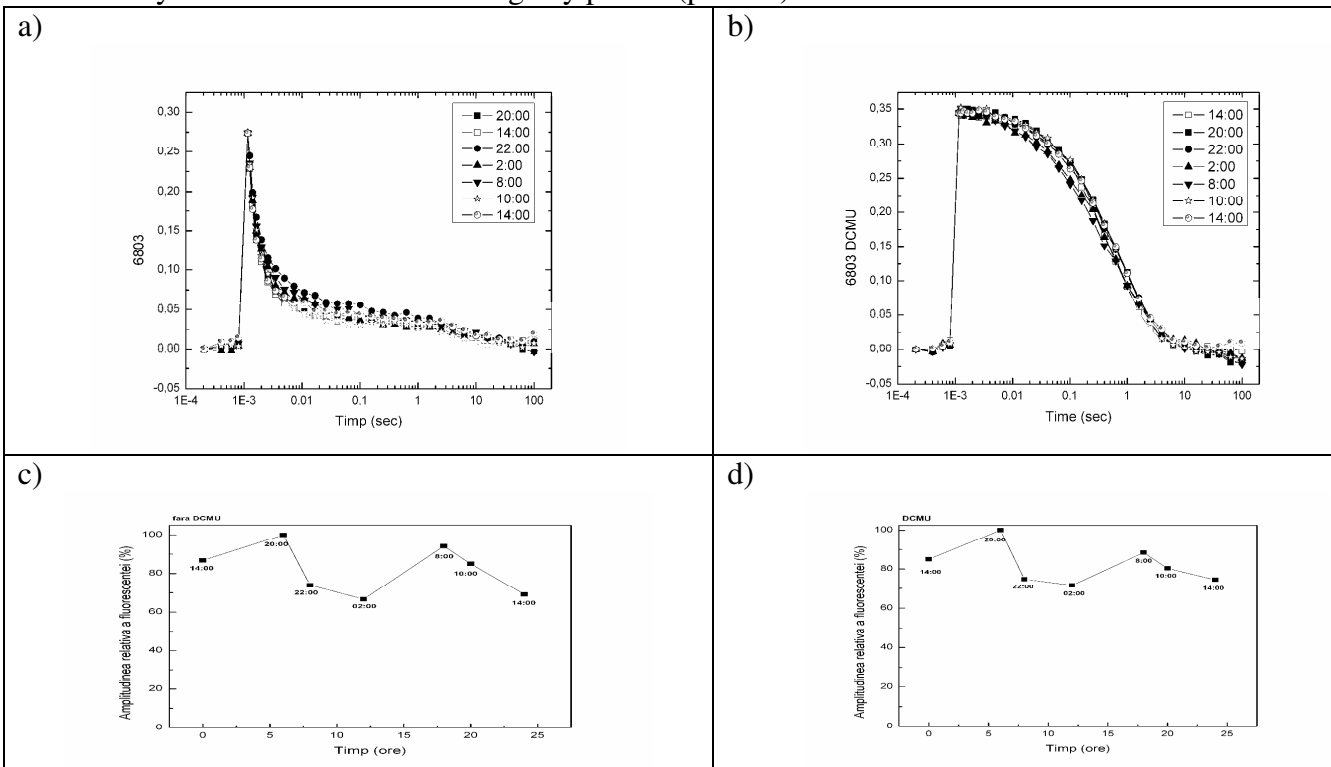

Figure 6. The effect of the circadian rhythm on the electron transport chain of PSII in both the acceptor part (panel a) and the donor part (panel b) of the electron transport chain of PSII. The effect of light-dark cycle on the relative amplitude of fluorescence in the absence (panel c) and presence (panel d) of DCMU.

Thus, a delay in the electron transfer in the acceptor part can be observed (panel a), which is correlated with a decrease of approximately 35% in the active centers number (panel b), followed by a total comeback of the number of active centers the next morning, during light period. This effect is correlated with the effect on the donor part of the electron transport chain (panel c).

In conclusion, a great change can be observed in both the donor and acceptor parts of PSII of *Cyanothece* ATCC51142, during the dark period. Our next studies will investigate the correlations between these modifications and a new isoform of D1 protein, as well as the D1 protein functionality during circadian rhythm.

3. Measurements on the function of photosystem II of cyanobacteria communities at the Ciocaia spring, Bihor.

The purpose of the project is the study of the functional diversity of D1 protein in both, cyanobacteria with a sequenced genome (model cyanobacteria strains) and cyanobacteria from cyanobacteria communities; therefore we optimized the protocol for the measurement of chlorophyll fluorescence in cyanobacteria communities, outdoor. This way, we were able to receive real time information regarding the acceptor part of the electron transport chain. One of the chosen locations for this study is in Ciocaia, Sacuieni, Bihor district, where there is a hot spring by drilling with a surface exit around which a high temperature (35ºC) adapted cyanobacteria crust formed (Figure 7). Samples were collected from these cyanobacteria crusts, as follows: on our first trip there we collected 10 samples, on our second trip we collected 14 samples and on our third trip we collected 12 samples during morning and afternoon, just in case some modifications of day-night cycle occur. From the samples taken we plan to isolate RNA, DNA, proteins and to sequence the genome and transcriptome, in order to observe the total number of functional copies of the *psbA* genes, as well as the *psbA* genes expressed in the cyanobacteria communities in certain environmental conditions.

Figure 7. The hot spring from Ciocaia. a) cyanobacteria communities at Ciocaia, b) outdoor measurements at Ciocaia, c) samples taken from the cyanobacteria community at Ciocaia.

Therefore, the studies undertaken by us complete the international efforts of understanding the functional diversity of D1 protein in order to make a possible correlation between different isoforms of D1 and the ecophysiological environment.

Date: 12 October 2013

Project director,
C.S.I. Dr. Cosmin Ionel Sicora