Babes-Bolyai University

Faculty of Biology and Geology

Doctoral School of Integrative Biology

Doctoral Thesis

Study of D1 protein function from photosystem II in cyanobacteria

-Summary-

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Key words: Photosystem II, *psbA* gene, Cyanobacteria, Photosynthesis Research, D1 protein

Aim of the study

The aim of these phD thesis consists in a detailed study of D1 protein function at cyanobacteria, to achieve this aim we formulated the next scientific objectives:

(i) to describe the D1 protein isoforms diversity from cyanobacteria and to analize their way of genetic expression, through an informatical analysis of the *psbA* gene sequences available in the data bases; (ii) a molecular and phisiological research of the D1 protein isoforms and their function in maintaining a functional photosystem II in cyanobacteria; (iii) to identify some environmental factors with effect on the D1 protein isoforms expression at cyanobacteria and (iv) experimental identification of new D1 protein isoforms, uncharacterized functional until now.

I. Introduction

The shortest definition for cyanobacteria would be that of prokaryotic organisms able to perform photosynthesis. They are great model organisms for photosynthesis research, being able to perform a complex process similar to higher plants, having the advantage that grow faster that the higher plants, they have growth conditions easier to maintain and are easier to manipulate by genetic techniques. The first photosynthetic organism whose genome was completely sequenced was the cyanobacteria *Synechocystis* sp. PCC 6803 (Kaneko et al., 1996).

As living environment, we have to highlight the fact that cyanobacteria have colonized all the environments, the only condition is the presence of light, so that we find them from fresh waters to marine ones, from the arctic desert until on the top of the mountains we can find cyanobacterial communities (Pepper et al., 2015). The first photosynthetic organisms, as oxygenic cyanobacteria or the anoxygenic photosynthetic

bacteria do not possess cellular organelles and the photosynthetic process take place inside the cells, in the tilakoid membranes (Govindjee et al., 1986).

Cyanobacteria are able to perform an extremely performing photosynthesis process, between 450-655 nm; they absorb light through a protein family strongly coloured, named phycobiliproteins (Glazer et al., 1986). They join together forming phycobilisomes, linked to uncoloured linker proteins on the external side of the photosynthetic lamella (Sonani et al., 2016). Phycobilisomes are protein complexes which consist in water soluble proteins which have covalent attached cromophores and phycobilins which give to cyanobacteria the characteristic colour blue-green (Sidler, 1994). They transfer the excitation energy to photosystem II, being able to transfer it also to photosystem I, in a process of short time setting of the process of capturing the photosynthetic energy that serves to adjust the excitation energy distribution to the reaction centres from both photosystems (van Thor et al., 1998). The phycobilisomes architecture allow them to absorb visible light between 500-670 nm, the energy transfer inside the phycobilisome can act as a protective mechanism which prevent the degradations of the photosystems by the excess of light energy (Liu et al., 2008). The light energy captured by the phycobilisomes is than fast transferred to the photosystems II and I.

Photosystem II is a protein complex from the tilakoid membrane which perform oxygenic photosynthesis by a range of electron transfer reactions induced by light which have as a result the water molecules cleavage in protons and molecular oxygen. At cyanobacteria, photosystem II is formed by 17 transmembrane proteins, 3 peripheral proteins and a number of cofactors with a total molecular weight of 350 kDa (Umena et al., 2011). The biosynthesis cycle of photosystem II is a dynamic one,

which starts in the cytoplasm membrane and continues in the the thylakoid membrane.

The core of the reaction centre from photosystem II is represented by the proteins D1 and D2, which bind all the redox active components, such as: the primary donor – chlorophyll P680, the primary acceptor, pheophytin, the secondary donor, the tyrosine Yz and all the primary and secondary acceptors, the Q_A and Q_B quinones. The primary charge separation in PS II results in chlorophyll P680 cations which are oxidized, they are the only elements strong enough to trigger the water oxidation. The recombination of chlorophyll cation P680 with downstream electron transport cofactors pheophytin or the primary stable electron acceptor plastoquinone Q_A can lead to the formation of triplet Chl states and ultimately to the formation of singlet oxygen, which in turn may damage the photosynthetic machinery, specially the D1 protein from the reaction centre (Mulo et al., 2009). The defence mechanisms acts by replacing the D1 protein with a new synthetized copy, at every 5 hours in normal light intensity or at 20 minutes in highlight intensity conditions (Tyystjarvi et al., 1994). The D1 protein is encoded by a small family of genes ranging from 1 to 6 members and whose expression must be under strict control to assure a functional photosynthetic process during the changes in the environmental conditions.

After the damage of the D1 protein from photosystem II by different environmental factors, the defense mechanism of PS II occurs. After partial disassembly of PSII, the damaged D1 protein is accessed by the FtsH protease, and degraded. Subsequently, the ribosome-nascent D1 chain complex is targeted to the thylakoid membrane, and the D1 protein is co-translational inserted into the membrane and the PSII complex. The C-terminus of the D1 protein is post-translational processed, PSII is re-

assembled, activated, and the PSII dimers are formed (Mulo et al., 2009). The presence of multiple *psbA* genes which encode different isoforms of D1 protein offers a clue on the importance these genes have in regulatory processes in order to maintain a functional photosystem II. There regulation processes follow at least two distinct strategies, one is to replace the existent D1 protein from PS II reaction centres with another isoform when stress conditions are detected (Oquist et al., 1995) the other involves the increase of the synthesis rate of the same D1 protein when stress conditions are detected. These two strategies have been described at many cyanobacterial strains, recently another defense mechanism was described, when during normal growth condition microaerobiosis (Sicora et al., 2009) or a decrease in oxygen level occurs (Summerfield et al., 2008).

Functional, the D1 protein isoforms from cyanobacteria can be classified in:

A. $D1_m$ or D1major

B. D1:1

C. D1:2

D. D1'

E. D1 *rogue* or D1 *sentinel*

A. D1m is a D1 form expressed and present in the PSII centres under normal growth conditions. D1m is also induced under most stress conditions (Mulo et al., 2009)

B. D1:1 is a D1 form expressed and assembled into PSII under normal growth conditions, but repressed under stress;

C. D1:2 is a D1 form repressed under normal growth conditions, but induced and accumulated into PSII by stress. Thence, during stress

conditions the D1:2 isoform is strongly induced, replacing the D1:1 isoform in the cells.

D. D1' is a D1 form "silent" under standard growth conditions, but induced under microaerobic/ low oxygen conditions.

E. D1 *rogue* or D1 *sentinel* is a D1 protein isoform highlighted in the last years. In 2012 its existence has been reported after *in silico* analysis was performed (Murray, 2012). This form is associated with diazotroph cyanobacteria; it blocks photosystem II during night allowing the nitrogen fixation.

In the last years, the understanding of the photosystem II defense mechanisms under different stress conditions and also the regulation of photosynthesis and respiration processes have captivated the attention of the researcher in all the world.

II. Materials and Methods

Cyanobacterial strains used in the present study were obtained from Pasteur Culture Collection (PCC, France) and also from Biological Research Institute from Cluj-Napoca. These are: *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7002, *Cyanothece* sp. PCC 8801 and *Anabaena variabilis* ATCC 29413 obtained from PCC France respectively the strain *Cyanothece* sp. ATCC 51142 obtained from Biological Research Institute, Cluj Napoca.

***Synechocystis* sp. PCC 6803** is the first cyanobacteria with a full genome sequenced and the data available in Cyanobase. The *psbA* gene family contains 3 genes; differently expressed according environmental conditions (Jansson et al., 1987).

***Synechococcus sp*. PCC 7002** is also a model cyanobacterial strain with a completely sequenced genome, unicellular, sometimes can form short filaments of 2-4 cells, the optimal growth temperature is 38°C.

The *psbA* gene family contains 3 genes, different expressed according to environmental conditions.

***Cyanothece* sp. ATCC 51142** is a unicellular cyanobacterium which has a light/dark cycle in 2 stages. During the day performs photosynthesis, and during night it performs nitrogen fixation. The D1 protein is represented by 5 *psbA* genes, which encode 4 different D1 isoforms, one of them is a hypothetical D1*rogue* isoform (Murray, 2012).

***Anabaena variabilis* ATCC 29413** is a filamentous cyanobacteria, which forms heterocysts, cells specialized where the nitrogen is fixed in the filaments grown in high oxygen level medium. The *psbA* gene family contains 5 genes (Park et al., 2013).

***Cyanothece* sp. PCC 8801** is a unicellular diazotroph cyanobacterium. Its cells contain also phycoerythrin, a pigment which allows the light absorption from a range of the spectra unused by many other photosynthetic organisms. It has 4 *psbA* genes which encode different D1 protein isoforms, one of them is a hypothetical D1*rogue* isoform (Murray, 2012).

Strain 7002 mutant was received from our co-workers from Berlin, from the research group of Professor Holger Dau, from Dr. Yvonne Zilliges, it is a cyanobacteria with a deletion of the gene *a2164*, which encodes the D1' isoform of D1 protein.

Growth Conditions

The cyanobacterial strains *Cyanothece* sp. ATCC 51142, *Anabaena variabilis* ATCC 29413, *Synechococcus* sp. PCC 8801 and *Synechocystis* sp. PCC 6803 were maintained in Erlenmeyer glasses, approximately 300 ml/glass, in a climatic chamber, grown in continuous light conditions, at a light intensity of 50 µmol photons $m^{-2}s^{-1}$ (50µE), at a constant temperature of 30 °C, with 130 RPM continuous shaking.

The *Synechococcus* sp. PCC 7002 and the mutant *Synechococcus sp*. PCC 7002 (ΔA2164) strains, termo-tolerant cyanobacteria strains were maintained in cylindrical tubes, approximately 300 ml/tube, in a bioreactor we made, grown in a continuous regime, with a light intensity of 50μE, at 38 °C constant temperature, bubbled with air.

The main research methods performed

- **Methods to investigate gene expression** –Total cyanobacterial RNA isolation using Trizol, cDNA synthesis with the iScript cDNA synthesis kit from BioRad and cDNA amplification using the (Q-RT-PCR technique, in an IQ5 analyser from BioRad, using specific primers for each isoform of D1 protein.

- **Methods to investigate the photosystem II function** through the chlorophyll fluorescence measurement technique. The fluorescence was measured with a double-modulation FL-3500 fluorometer. Chlorophyll fluorescence measurement are performed to observe the electron transfer in the donor and acceptor side of the electron transport chain and also to see the number of active centres / potentially active centres at the moment of light flash. The transfer between the fixed quinone Q_A and the mobile one Q_B takes place in microseconds until one millisecond and can be measured with a fluorometer.

- **Methods to detect and quantification of D1 protein,** involve protein isolation using a previously described protocol by Brown et al., 2008, protein electrophoresis in polyacrylamide gel (SDS-PAGE) and Western Blotting techniques using a general antibody for D1 proteins with specificity for the C-terminus end from Agrisera- AS01016.

- **Methods of bioinformatic analysis -** all *psbA* gene sequences were obtained from Cyanobase data base, and imported in the software CLC Sequence Viewer 6.9.1. Using the aminoacid sequences we

performed a multiple alignment of all the 91 sequences, based on progressive alignment algorith. We than edited the alignment, the non-informative areas were deleted, resulting sequences of 360 positions. This made posible to compare the highly conserved aminoacids at all D1 isoforms. The next step consisted in the development of a Neighbour Joining phylogenetic tree, 1000 replicates, with the CLC Sequence Viewer 6.9.1. software.

Tests applied to cyanobacterial strains

- Study of high light intensity influence on the PS II function – the cianobacterial cultures were grown at a 50 µE light intensity, than we applied 600 µE light intensity during the treatment, after that we went back at the initial conditions during the recovery period, for 60 minutes.

- Evaluation of UV-B radiation influence on PS II function– the cianobacterial cultures were grown at the 50 µE light intensity until a chlorophyll concentration of 6 µg chl ml^{-1} ($D.O._{580\ nm}$ =1), corresponding to the exponential growth phase of the cells. Over the initial growth conditions we applied a UV-B radiation treatment at an intensity of 0,307 (mW/cm^2), than we returned at the initial growth conditions during the recovery period.

- The effect of microaerobic condition on D1 protein isoform expression and on PS II function – the treatment was applied by bubbling N_2 during the 120 minutes treatment, than we returned the initial growth conditions for 60 minutes, corresponding to the recovery period.

- Evaluation of PS II function changes during light/dark cycles – cyanobacteria were grown in a 12hour light / 12 hour dark cycle at 30 ºC and 50 µE light intensity until a chlorophyll concentration of 6µg chl ml^{-1}, corresponding to exponential growth phase of the cells. The

experiments were performed by samples taken hour by hour at every 60 minutes in absence and presence of DCMU.

- Study of CO_2 influence on PS II function – to cells grown in normal growth conditions were performed 3 types of experiments: to cells grown in atmospheric air were induced microaerobic conditions for 120 minutes by N_2 bubbling, cells grown with a gas mix of 5% CO_2 and 95% air were treated with atmospheric air (with a CO_2 concentration of 0, 03 - 0, 04%), and cells grown with atmospheric air were treated with synthetic air, without CO_2 for 48 hours.

III. Results and Discussion

III.1. Bioinformatic analysis of D1 protein diversity at cyanobacteria

The aim of this research was to systematize, using some bioinformatical tools, the available data about the products resulted from the *psbA* genes translation from different cianobacterial species, using the sequences available in the special database Cyanobase (http://genome.annotation.jp/cyanobase). Also, we intended to propose some evolutionary strategies for the evolution of *psbA* gene family.

After the analysis of 90 aminoacid sequences resulted from the *psbA* translation, which encodes different isoforms of D1 protein at 30 cyanobacterial strains with completely sequenced genome, we established a distribution of the genes at different species and also identified the conserved aminoacids specific for every isoform encoded by *psbA* genes.

For the D1:1 isoform, characteristic is a substitution of the glutamic acid (E) by a glutamine (Q) in position 130 (Campbell et al., 1998) From the 30 cianobacterial strains studied, 27 have genes which encode potential the D1:1 isoform.

For the D1:2, it is documented the most conserved change is in the position 130. At this position, it is conserved a glutamic acid (E) instead of a glutamine (Q) at 17 from the total of 30 cianobacterial strains studied. At these ones, we observed 45 *psbA* genes which potentially encode the isoform D1:2.

At the D1' isoform, we identified the protein sequences which have the 3 changes in the aminoacid structure characteristic to this isoform, in the positions 80, 158, 286 (Sicora et al., 2009). From the analysis of the sequence data available for the 30 strains, 10 strains have genes which encode potential the isoform D1'.

For the D1 *rogue* isoform Murray (2012) concluded that hypothetical there is a new isoform of D1 protein which blocks the PS II during night, allowing nitrogen fixation. The D1 *rogue* isoform has 6 specific conserved changes in the aminoacid structure. After the analysis of all the sequences from the 30 cianobacterial strains with sequenced genome, we observed 3 strains whose protein sequences have 3, 4 or 5 changes in the aminoacid sequences characteristic to D1 *rogue* isoform.

The alignment of the 90 aminoacid sequences constituted the base for a phylogenetic tree using the Neighbour Joining algorithm, 1000 replicates. After our analysis, it can be concluded that there are 2 possible ways in which *psbA* genes and the D1 protein encoded by them have evolved. The first strategy involves an horizontal gene transfer, the second strategy involves duplication of the existing genes and spontaneous mutagenesis of duplications, followed by a selection, as a need to adapt at new environmental conditions.

III.2. Effect of microaerobic conditions on D1 protein isoform expression and on PS II function at model cianobacterial strains.

Cyanobacteria strains face low oxygen conditions in different habitats like thermal springs or in case of specialized cell like heterocyst where the photosynthesis is limited (Voorhies et al., 2012). Extremely important for understanding the complex photosynthetic process is the adaptation of the cells at this conditions and their transition to aerobic conditions (Summerfield et al., 2011).

The first experiment was performed applying a microaerobic stress to the cyanobacteria *Synechocystis* sp. PCC 6803, at this strain there is an induction effect described before (Summerfield et al., 2008; Sicora et al., 2009), of the *psbA1* gene in microaerobic conditions. At this strain we observed also in chlorophyll fluorescence studies an effect on the donor side of PS II, displayed by acceleration of middle phase on the reoxidation curve.

At *Synechococcus* sp. PCC 7002, the microaerobiosis treatment didn't show a major effect on *psbA* isoforms expression (Figure 1), even if this strain has the characteristic aminoacid changes conserved for the D1' isoform. The unchanged expression of the *psbA3* gene during the microaerobic treatment remains a unique aspect of our experiments.

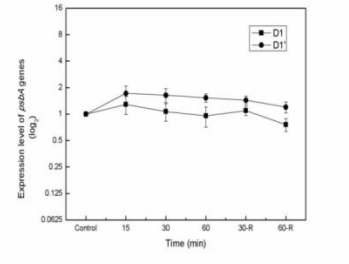

Figure 1. Microaerobiosis effect on *psbA* gene transcription at *Synechococcus* sp. PCC 7002.

III.3. Effect of oxidative stress on PS II function at model cyanobacterial strains.

III.3.1. Influence of light intensity variations on PS II function at model cyanobacterial strains.

A demand of all the organisms able to perform photosynthesis is to adapt at changes of different light intensities in their growth environment. The acclimation process includes changes in the photosynthetic mechanism, specially the degradation and replacement of the D1 protein from the PS II reaction centre (Muramatsu et al., 2012).

In our experiments, we analysed the effect that the passing from the basic light intensity to a high light intensity has on the PS II function. The aim of the experiments was to understand the functional diversity of D1 protein in changing environmental conditions.

The cianobacterial strains studied were *Synechococcus* sp. PCC 7002, *Cyanothece* sp. ATCC 51142 and *Anabaena variabilis* ATCC 29413. In what concerns the cyanobacteria *Synechococcus sp.* PCC 7002, it was highlighted an effect of high light intensity which manifested through a decrease in the number of active centres until 65% followed by a recovery until 70% in absence of DCMU, respectively a decrease until 50% followed by a recovery until 70% of the total number of centres available in PS II in presence of DCMU. In absence of DCMU, can be seen an alteration of the middle phase on the fluorescence curve, corresponding to the electron transfer from Q_A^- to Q_B with the Q_B pocket empty at the moment of the light flash, showed by a fast electron transfer in the acceptor side of PS II. In presence of DCMU, there are not major changes on the fluorescence curve showing the fact that the high light intensity doesn't have an effect on the electron transfer in the donor side of PS II.

III.3.2. Influence of UV-B radiation on PS II function at model cyanobacterial strains.

The cianobacterial strains studied at which we analyzed the effect of UV-B radiations were *Synechococcus* sp. PCC 7002, *Anabaena variabilis* ATCC 29413 and *Cyanothece* sp. ATCC 51142. In what concerns the UV-B radiations effect on the cyanobacteria *Synechococcus sp*. PCC 7002 (Figure 2), in absence of DCMU, it is expressed a decrease in the electron transport between Q_A and Q_B, in the middle fast phase, decrease reversible in the recovery period and more visible at 60 minutes of treatment. It is for the first time when this effect is visible at a cyanobacterial strain during the UV-B treatment which, at other cyanobacteria like *Synechocystis* sp. PCC 6803 or *Gloeobacter violaceus* PCC 7421, do not induce chages in the acceptor side of photosystem II in similar experimental conditions (Sicora et al., 2008; Vass et al., 1999).

In presence of DCMU is visible an acceleration of electron transport in the donor side of photosystem II, showing a recombination of Q_A^- with co-factors closer from the electron transport chain, cause by an inhibition of water oxidation complex (Sicora et al., 2003).

It was also observed a decrease in the number of active complexes of photosystem II and also of the photosynthesis process efficiency of 40 % followed by a recovery of 10% in the absence and presence of DCMU.

The acceptor side of photosystem II at cyanobacteria *Anabaena variabilis* ATCC 29413 is affected by the UV-B radiations, specially the electron transfer between the quinones Q_A and Q_B, but was also evident a decrease in the number of active centres of photosystem II, in absence and presence of DCMU. In what concerns the cyanobacteria *Cyanothece* sp. ATCC 51142, the UV-B radiations have a specific effect, documented

before, on the donor side of PS II (Vass et al., 1999; Vass et al., 2000; Sicora et al., 2003; Sicora et al., 2006).

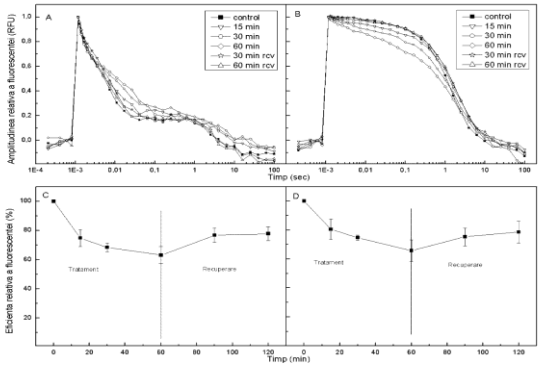

Figure 2. Effect of UV-B radiation on the acceptor and donor side of PS II at *Synechococcus* sp. PCC 7002.

III.4. Influence of CO_2 concentration on photosystem II at cyanobacteria

Cyanobacteria, in control with the atmospheric oxygen formation and also being at the origin of the plastid, evolved different strategies to adapt at different environmental conditions and also at different levels of CO_2 from the environment. This complex process of adaptation includes changes in gene expression involved in carbon concentration mechanism.

The aim of our experiments was to analyse how the *Synechocystis* sp. PCC 6803 strain cells act during 3 types of experiments, described in the chapter Materials and Methods.

Considering that previous studies (Sicora et al., 2008; Summerfield et al., 2009) showed that the *psbA1* gene is induced when oxygen is removed and concluded that the effect is due to oxygen we

intended to remove the other gases from the air composition (O_2 and CO_2) and to observe their individual effect. It was distinguished, from our experiments, that the cyanobacterial strain treated with microaerobic condition showed a strong effect of *psbA* gene, as previously shown (Summerfield et al., 2008; Sicora et al., 2009). When returning to initial growth conditions, the expression level decreases until at the initial level.

Starting from the idea that, during microaerobiosis, bubbling the growth medium with nitrogen in order to remove O_2 could remove also CO_2 and induce the *psbA1* gene expression it was presumed that we should see an induction of the gene independent on O_2 concentration. To test this hypothesis, the cells grown with 5% CO_2 were subjected to a treatment with synthetic air, composed only from oxygen and nitrogen and we observed the *psbA1* gene induction effect. In this case, contrary to what we expected, the effect of *psbA1* gene induction does not exist.

The experiment of passing the cells from high CO_2 concentration to atmospheric level was realized by bubbling the culture initially with 5% CO_2 until de optical density of the culture at 730 nm was 0.8, than the air was replaced by atmospheric CO_2, which means a low level for the next 120 minutes (Figure 3). When passing the cells to low CO_2 concentration the *psbA1* gene showed a strong induction of 30 times in the first 15 minutes of treatment and remained induced all the 120 minutes during the atmospheric air bubbling. It is obvious also a decrease of gene expression after the treatment the level remaining above the control level. What is interesting is that in the recovery conditions, when the cells were subjected again to the gases with 5% CO_2, the *psbA1* gene expression continues to grow.

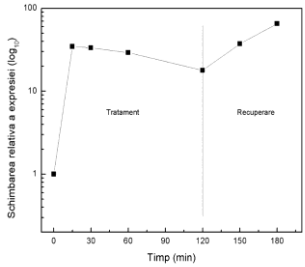

Figure 3. *psbA1* gene expression quantified by RT-PCR technique, in cells grown with 5% CO_2 and transferred to atmospheric air.

Next, we wanted to search the existence of a regulatory element involved in *psbA1* gene expression control, dependent on CO_2 level. We analysed the promotor sequence of the *psbA1* gene. NdhR, a LysR type regulator, which control the expression of most of the Ci responsive gene in *Synechocystis* sp. PCC 6803 by binding to a signature sequence, showed by a T-N11-A type motif (Figge et al., 2001). Indeed, the research of *psbA1* gene sequence revealed a similar palindromic sequence, indicating the conceivable regulation by NdhR under low CO_2 condition.

III.5. Functional role of D1`protein in response to light stress, UV or hypoxic stress at cyanobacteria

In order to highlight the functional role of D1`isoform, we performed experiments on 2 cyanobacterial strains, *Synechococcus* sp. PCC 7002 wild type and a mutant strains which has a deletion of the gene which encodes the D1`of D1 protein, respectively a2164, named from now "7002 mutant". To test if the strain *Synechococcus* sp. PCC 7002 has a potential D1` isoform of D1 protein, we analysed the genetic sequences from this strain from Cyanobase. The alignment was performed using CLC Sequence Viewer 6.9.1 software. *Synechococcus* sp. PCC 7002 has 3 *psbA* genes which encode different isoforms of D1 protein, *psbA1*,

psbA2 and *psbA3*. It was observed the presence in the aminoacid sequence of the protein SYNPCC7002_A2164 of all the 3 substitutions characteristic to D1` isoform of D1 protein.

Next, we wanted to investigate the possible changes in the absorption spectra of the photosynthetic pigments at both cyanobacterial strains. It is evident that the mutant strain of *Synechococcus* sp. PCC 7002 has better chlorophyll to phycobiliproteins ratio, associated with a higher capacity to absorb the light (Figure 4).

Then both cyanobacterial strains were subjected to different types of stress, to see how the act in these new environmental conditions. During the high light intensity treatment it was obtained a better recovery ability of the mutant strain at 120 minutes of applied treatment especially in the absence of DCMU. The experiments didn't reveal any notable differences between the wild strain and the mutant one during the UV-B or microaerobic treatment. During the recovery period after the environmental stress (by light, UV-B radiation or microaerobiosis), it was detected a higher quantity of total D1 protein, a typical effect for a cellular reaction after a stress period.

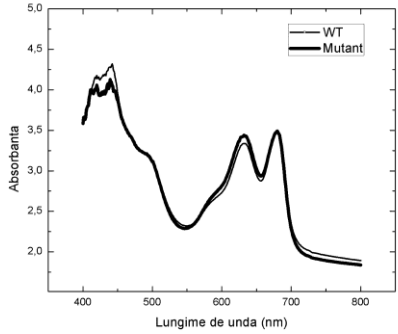

Figure 4. *In vivo* absorption spectra of the cyanobacterial strains *Synechococcus* sp. PCC 7002 wild type and mutant.

III.6. Functional changes in PS II during light/dark cycles

The main goal of our studies presented in this chapter was observing the way in which occurs modification of the donor and acceptor side of photosystem II during a cycle of 12 hours light/12 hour dark, for a better understanding of the mechanism involved in the adaptation of the cells at these conditions. Another objective was to explore the possible involvement of D1 *rogue* isoform in this mechanism as a way to respond at the variations in the quantity of light. The strains used in the study were *Cyanothece* sp. ATCC 51142, *Anabaena variabilis* 29413, *Cyanothece* sp. PCC 8801 and *Synechocystis* sp. PCC 6803. We performed chlorophyll fluorescence measurements. The cyanobacterial strains were grown in cycle of 12 hours light 12 hours dark. The experiments demanded sampling for chlorophyll fluorescence measurements every 60 minutes during 26 hours, in absence and presence of DCMU. The next step consisted in checking if the modifications occurred in the donor or acceptor side or dependent on protein synthesis or not, so that we added in the growth medium lincomycin, an inhibitor who blocks the synthesis of new proteins (Vazquez, 1974).

Regarding the cyanobacterial strain *Cyanothece* sp. ATCC 51142, we observed a deceleration in the electron transfer between Q_A and Q_B, very obvious in the centres with Q_B pocket free at the moment of flash. The change was visible at 15 minutes after the dark conditions appeared and was reversible at 15 minutes after the light turned on, the cells were totally recovered at around one hour after the light was restored in the climatic chamber.

The occurrence of this fast phase also in the donor side of the electron transport chain strongly suggests an inhibition of the water

oxidation complex. This change appears after the dark condition appears and it is perfect reversible at the start of light.

In what concerns the number of active centres of photosystem II, we see that the changes in the donor and acceptor side are correlated with a decrease in the number of active centres until 60% during night followed by a recovery in the number of active centres when the light was turned on, both in absence and presence of DCMU. The decrease in the number of active centres during night suggests an inhibition of photosystem II during the dark conditions.

In order to investigate if the changes appeared in the donor or acceptor side of PS II at *Cyanothece* sp. ATCC 51142 are dependent by protein synthesis, it was added, before the dark condition, lincomycin in the growth medium. In presence of lincomycin we saw a smaller effect in the donor and acceptor side than in the absence of it.

All the three cianobacterial strains which have a possible D1 *rogue* isoform of D1 protein, present changes in the acceptor side of photosystem II, especially at the recombination of the quinone Q_{A-} with centres which have a Q_B situs at the moment of light flash. This observation suggests a strong modification of photosystem II during the dark phase.

The control strain used, *Synechocystis* sp. PCC 6803, doesn't present any changes in the donor or acceptor side of PS II, being able to maintain a functional photosystem II during the dark.

IV. General Conclusions

a. The bioinformatic analysis of *psbA* gene sequences from data bases allowed the highlight of some specific changes in the aminoacid structure conserved at every isoform of D1 protein.

b. After the microaerobic stress applied to *Synechococcus* sp. PCC 7002, we concluded that this strain contains an unspecific form of D1 protein, uncharacterized until now.

c. Another novelty element of this doctoral thesis is the atypical effect that UV-B radiations have on the acceptor side of photosystem II in *Synechococcus* sp. PCC 7002, efect not shown until now.

d. Studies on *Synechocystis* sp. PCC 6803 showed that both O_2 and CO_2 act as factors that trigger the induction of *psbA* gene expression, by independent mechanisms. Another novelty of this research is the presence in the *psbA1* gene structure of a regulatory element of expression under CO_2 as a trigger factor.

e. It was demonstrated that the strain *Synechococcus* sp. PCC 7002 mutant has an increased efficiency in PS II repairing after the damaging effect of the stress factors, probably by triggering some internal protection mechanisms in order to respond at stress conditions. This effect has to be investigated supplementary further.

f. Studies on the effect of light dark cycles on the photosystem II function at *Cyanothece* sp. ATCC 51142 showed that the changes occured in donor and acceptor side of photosystem II depend partially on *de novo* protein synthesis.

Thence, after the systematization of the data available in Cyanobase data base, we identified the specific conserved aminoacids for each isoform of D1 protein and we proposed some evolutionary models of the *psbA* gene family. After the microaerobiosis experiments, we

identified an atypical, form of D1 protein at *Synechococcus* sp. PCC 7002. At this strain it was also observed also an undocumented before effect of UV-B radiation on the acceptor side of PS II. Studies on *Synechococcus* sp. PCC 6803 showed an independent effect of O_2 and CO_2 in triggering *psbA1* gene expression and also the existence of a regulatory mechanism of expression under CO_2 conditions. After our experiments, it was highlighted an effect of mutant *Synechococcus* sp. PCC 7002 strain of better recovery after the damaging effect of environmental factors, another novelty is presented by the fact that the changes in donor and acceptor side of PS II at *Cyanothece* sp. ATCC 51142 are partially dependent by *in vivo* protein synthesis.

These studies lend to a more complex image of physiology and function of PS II and open the way for new research approaches in order to clarify the aspect unknown yet.

V. References

Campbell D, Eriksson MJ, Öquist G, Gustafsson P, Clarke A. 1998, The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction-center D1 proteins. *Proc Natl Acad Sci USA* **95**, 364-369.

Chiș C, Drugă B, Carmel D, Chiș I, Ardelean A, Sicora CI. 2016, UV-B stress changes the electron flow on photosystem II complex in *Synechococcus* sp. PCC 7002. *Rom Biotechnol Lett* **22**, 12142-12146.

Chiș C, Carmel D, Chiș I, Ardelean A, Dragos N, Sicora C. 2017, Expression of *psbA1* gene in *Synechocystis* sp. PCC 6803 is influenced by CO_2. *Open Life Sci* **12**, 156-161.

Glazer AN, Clark JH. 1986, Phycobilisomes: macromolecular structure and energy flow dynamics. *Biophys J* **49**, 115-116.

Govindjee, Amesz J, Fork DC. 1986, Light emission by plants and Bacteria. *Academic Press*, Orlando.

Kaneko T, Matsubayashi T, Sugita M, Sugiura M. 1996, Physical and gene maps of the unicellular cyanobacterium Synechococcus sp. strain PCC 6301. *Plant Mol Biol* **31**, 193-201.

Liu LN, Elmalk AT, Aartsma TJ, Thomas J-C, Lamers GEM, Zhou B-C, Zhang Y-Z. 2008, Light-induced energetic decoupling as a mechanism for phycobilisome-related energy dissipation in red algae: a single molecule study. *PLOS ONE* **3(**9): e3134.

Mulo P, Sicora CI, Aro EM. 2009, Cyanobacterial *psbA* gene family: optimization of oxygenic photosynthesis. *Cell Mol Life Sci* **66**(23):3697–3710.

Murray JW. 2012, Sequence variation at the oxygen-evolving centre of photosystem II: a new class of 'rogue' cyanobacterial D1 proteins. *Photosynth Res* **110**, 177–184.

Öquist G, Campbell D, Clarke AK, Gustafsson P. 1995, The cyanobacterium *Synechococcus* modulates Photosystem II function in response to excitation stress through D1 exchange. *Photosynth Res* **46**, 151-158.

Park J-J, Lechno-Yossef S, Wolk CP, Vieille C. 2013, Cell-specific gene expression in *Anabaena variabilis* grown phototrophically, mixotrophically, and heterotrophically. *BMC Genomics* **14**:759.

Pepper IL, Gerba GB, Gentry TJ. 2015, E*nvironmental Microbiology, third edition.* DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00001-6.

Sander J, Nowaczyk M, Kopczak M, Rogner M. 2008, Role of the *psbA* gene family of PSII from the thermophilic cyanobacterium *ThermoSynechococcus elongatus.* In: Allen JF, Gantt E, Golbeck JH,

Osmond B (eds) Photosynthesis. Energy from the Sun. *Springer, Dordrecht*, 745-748.

Sicora CI, Appleton SE, Brown CM, Chung J, Chandler J, Cockshutt AM, Vass I, Campbell DA. 2006, Cyanobacterial *psbA* families in Anabaena and *Synechocystis* encode trace, constitutive and UVB-induced D1 isoforms. *Biochim Biophys Acta (BBA)-Bioenergetics* **1**, 47-56.

Sicora CI, Ho F, Salminen T, Styring S, Aro EM. 2009, Transcription of a "silent" cyanobacterial *psbA* gene is induced by microaerobic conditions. *Biochim Biophys Acta* **1787**, 105–112.

Sidler WA. 1994, Phycobilisome and phycobiliprotein structures. In: The molecular biology of cyanobacteria. *Kluwer Academic Publisher, The Netherlands* 139-216.

Sonani RR, Rastogi RP, Patel R, Madamwar D. 2016, Recent advances in production, purification and applications of phycobiliproteins. *World J Biol Chem* **7**(1): 100-109.

Summerfield TC, Toepel J, Sherman LA. 2008, Low-oxygen induction of normally cryptic *psbA* genes in cyanobacteria**.** *Biochemistry* **47,** 12939–1294.

Summerfield TC, Nagarajan S, Sherman LA. 2011, Gene expression under low-oxygen conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 demonstrates Hik31-dependent and -independent responses**.** *Microbiology* **157**, 301–312.

Tyystjärvi T, Aro EM, Jansson C, Mäenpää P. 1994, Changes of aminoacid sequence in PEST-like area and QEEET motif affect degradation rate of D1 polypeptide in photosystem II. *Plant Mol Biol* **25**, 517–526.

Umena Y, Kawakami K, Shen J.-R, Kamiya N. 2011, Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9A *Nature* **473**, 55–60.

Van Thor JJ, Gruters OWM, Matthijs HCP, Hellingwerf KJ. 1999, Localization and function of ferredoxin: $NADP^+$ reductase bound to the phycobilisomes of *Synechocystis*. *EMBO Journal* **18**, 4128-4136.

Vass I, Kirilovsky D, Perewoska I, Mate Z, Nagy F, Etienne A-L. 2000, UV-B radiation induced exchange of the D1 reaction centre subunits produced from the *psbA2* and *psbA3* genes in the cyanobacterium *Synechocystis* sp. PCC 6803. *Eur J Biochem* **267**, 2640-2648.

Vass I, Kirilovsky D, Etienne A-L. 1999, UV-B radiation induced donor and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochemistry* **38**, 12786–12794.

VI. Scientific activity
Articles

Chiș C, Chiș I, Sicora O, Dalton C, Grec A, Ardelean A, Dragoș N, Sicora C. (2014) Comparative analysis of D1 protein sequences in cyanobacteria, *Studia UBB Biologia* **59** (2): 5-16.

Chiș I, Drugă B, Dalton C, **Chiș C**, Ardelean A, Dragoș N, Sicora CI. (2016) Effect of microaerobiosis on photosystem II in *Synechococcus* sp. PCC 7002. *Studia UBB Biologia* **61** (2): 197-204.

Chiș C, Drugă B, Dalton C, Chiș I, Ardelean A, Sicora CI. (2016) UV-B stress changes the electron flown on photosystem II complex in *Synechococcus* sp. PCC 7002**.** *Rom Biotechnol Lett* **22** (6): 12142-12146. (IF^{2016}=0,396; AIS^{2016}=0.083 – conf. InCites JCR Clarivate Analytics)

Chiș C, Dalton C, Chiș I, Ardelean A, Dragoș N, Sicora CI. (2017) Expression of *psbA1* gene in *Synechocystis* sp. PCC 6803 is influenced by CO_2. *Open Life Sci* **12** (1): 156-161.

(IF^{2017}= 0,764; AIS^{2017}=0,15 conf. InCites JCR Clarivate Analytics).

Chiș C, Chiș I, Sicora O, Dalton C, Grec A, Ardelean A, Dragoș N, Sicora C. (2014) Forward electron transport measured *in situ* in microbial mats from a hot spring in N-W Romania, *Studia UBB Biologia* **59** (2): 17-26.

Book Chapters

Chiș C, Mahboob A, Vassilieev S, Bica A, Peca L, Brouce D, Aro EM, Sicora C. (2013) D1' – a new member of D1 protein family in cyanobacteria, Photosynthesis: *Research for food, fuel, and future. Advanced Topics in Science and Technology in China*. Springer, Berlin, Heidelberg, pp. 358-360. https://doi.org/10.1007/978-3-642-32034-7_75 .