Scientific report

About the project implementation ***"Functional diversity of Cyanobacterial D1 proteins of photosistem II"*** *cod PN-II-ID-PCE-2011-3-0765 from January – December 2012*

After the primary implementation period that took place in 2011 when the preliminary activities have been done, in 2012 the following activities have been accomplished:

- The research team has been completed by employment of a postdoctoral fellow on the vacant position;
- The completion of major laboratory equipment acquisition by purchasing a specifically designed photobioreactor for cyanobacterial photosynthetic efficiency studies;
- Starting the studies about the functional characterization of D1 protein forms from model cyanobacteria that have the entire genome sequenced. From these experiments a big volume of data have been generated that will be reported in several scientific papers that are in preparation. A summary of these data is presented hereinafter;
- In this period, have been established collaboration relationship with other researchers and we were visited by 3 outstanding researchers in the cyanobacterial field from Biological Research Center Szeged, Imperial College London and Turku University Finland.

The cyanobacterial species studied in this phase are: *Anabaena variabilis* ATCC29413; *Cyanothece sp.*ATCC51142 and *Synechococcus sp*. PCC7002.

1. The effect of high-light intensity over the PSII function

We studied the influence of high-light intensity over the species *Anabaena variabilis* ATCC29413; *Cyanothece sp.* ATCC51142 and *Synechococcus sp*. PCC7002.

The cultures have been grown at 30ºC, 50μE light intensity, then were applied some treatments with 500 and 600 μE light intensity, in a tank of 10/10cm. We took samples for chlorophyll fluorescence measurements, for ARN and proteins. We illustrate here the results of the study of increasing light intensity influence over the photosystem II function at *Cyanothece sp.* ATTC51142.

Strain		Chlorophyll concentration (μg/ml)	Initial light intensity (μE)	Treatment Light intensity (μE)	Culture volume (ml)	Temperature (ºC)	Growth Media
Cyanothece sp. ATCC 51142	Exp.1	5.98	50	500	150	30	ASP2
	Exp.2	5.96	50	500	150	30	
	Exp.3	6.05	50	500	150	30	

Table 1. The experimental parameters used for light treatment over the strain *Cyanothece ATCC51142*

The fluorescence results indicate the effect of high-light intensity on the donor and acceptor side of photosystem II. Panels a and b (Fig.1) indicate the electron transport in the electron transporter chain of PSII, in absence (Fig1.a) and presence of DCMU (Fig1.b). **control** indicates the sample at 50μE, **15, 30, 60 min** indicates the samples at 15, 30, 60 minutes of 500 μE light treatment, and **30min rcv** and **60 min rcv** are the samples after a recovery of the cells at the initial light intensity, 50 μE.

Panels c and d (Fig. 1) show an average of the relative fluorescence amplitude out of 3 experiments in presence and absence of DCMU. We can see here a strong effect, a decrease of relative fluorescence amplitude with almost 50 percents during treatment, followed by a recovery with 30-40% during recovery period.

At *Anabaena variabilis,* although the fluorescence curves shape are not modified, it is obvious a decrease of fluorescence amplitude with 50% during treatment, followed by an increase with 10-15 percents during recovery time.

At *Synechococcus sp*. PCC7002, it is observed a decrease of fluorescence amplitude to 50%, followed by a recovery with 25-30% during recovery.

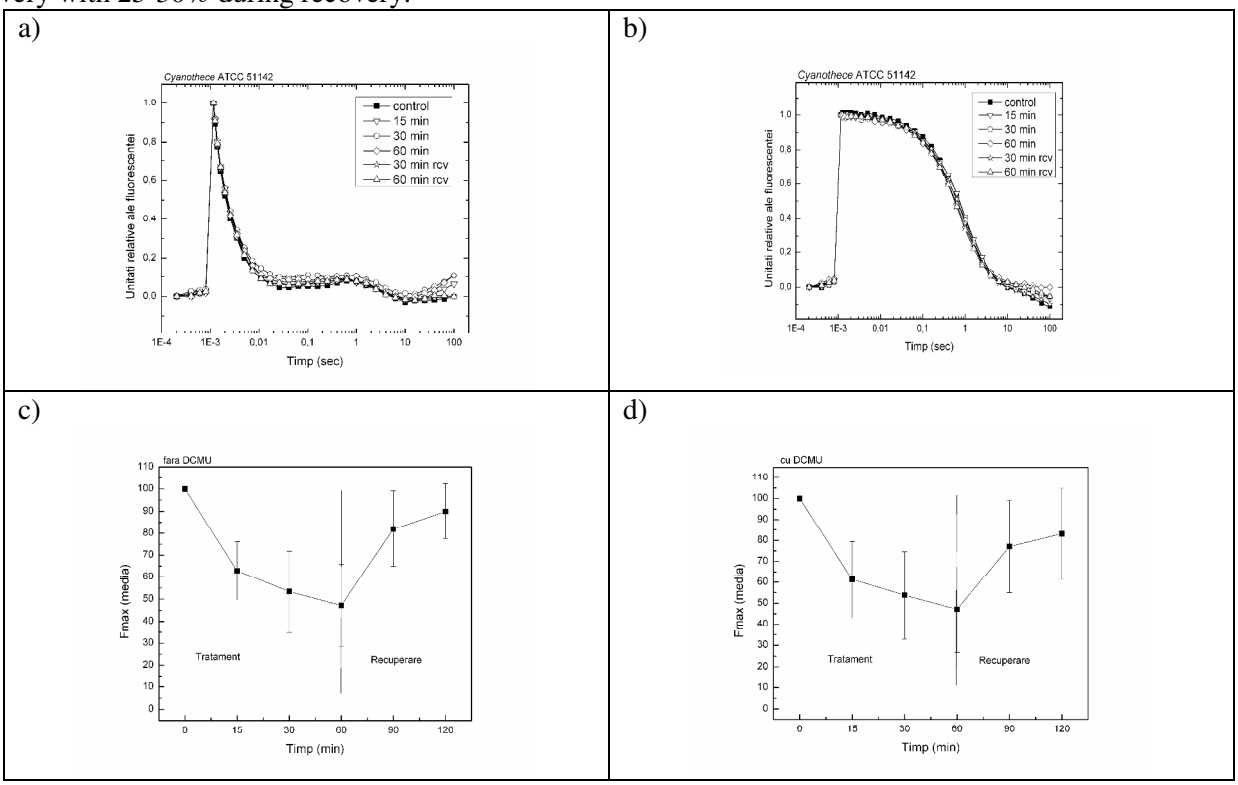


Figure 1. The effect of light intensity increasing over the photosystem II function at *Cyanothece sp.ATCC51142*

2. The study of UVB effect over the photosystem II function.

We studied the influence of UVB radiation increase over the strains *Anabaena variabilis* ATCC29413; *Cyanothece* sp.ATCC51142 and *Synechococcus sp*. PCC7002.

The cells grown at 50μE light intensity have been treated with UVB radiation (280-315nm). The initial UVB intensity was 0,737 mw/cm^2, during the treatment over the cells was applied a K_2CrO_4 (layer of 0,8cm), this layer reduced the UVB intensity at 0,307 mw/cm^2. We took ARN and protein samples, and also samples to measure chlorophyll fluorescence. To exemplify, we show here the results of the effect that UVB radiation have over the *Anabaena variabilis* ATCC29413 strain.

Strain		Chlorophyll Concentration (μg/ml)	Growth Intensity (μE)	UVB Intensity (mw/cm^2)	Culture volume (ml)	Temperature (ºC)	Growth Media
Anabaena variabilis ATCC 29413	Exp.1	6.02	50	0.307	200	30	BG11
	Exp.2	6.19	50	0.307	200	30	
	Exp.3	6.09	50	0.307	200	30	

Table 2. The experimental parameters used at the UVB radiation treatments over the strain *Anabaena variabilis* ATCC 29413

Panels a and b (Fig.2) indicates the electron flow through the photosynthetic electron transporter chain of photosystem II, in absence (Fig2.a) and presence of DCMU (Fig2.b). **Control** indicates the sample at the initial growth intensity, 50μE**; 15, 30, 60 min** are samples during the UVB treatment, **30min rcv** and **60min rcv** indicates the recovery time, moving the cells again at the initial light intensity, of 50μE;

Panels c and d (Fig. 2) show an average of relative fluorescence amplitude out 3 experiments in presence and absence of DCMU.

It is easy to observe in presence of DCMU an acceleration of electron transport speed (Fig2.b), and also a decrease of fluorescence amplitude at 70 percents during the treatment, followed by a recovery with 10 percents of the amplitude during the recovery time (Fig2. c, d).

At *Cyanothece* can be seen an increase in electron transport speed at the 60 minutes sample, and a decrease of relative fluorescence amplitude with 20-25% followed by a recovery during the recovery time.

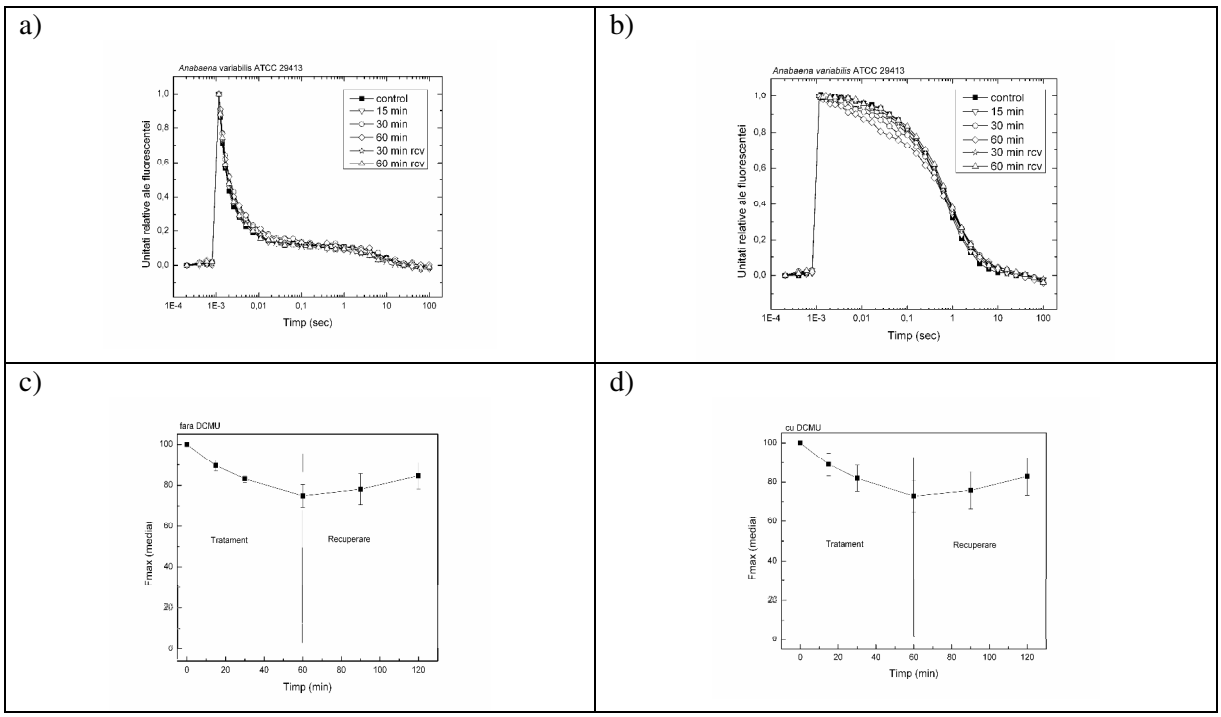


Figure 2. Influence of UVB radiation over the photosystem II function at *Anabaena variabilis* ATCC29413.

We also made some preliminary studies of D1 protein quantification using specific antibodies with the Western Blott method. We illustrate here an UVB treatment at *Synechococcus sp*. PCC7002.

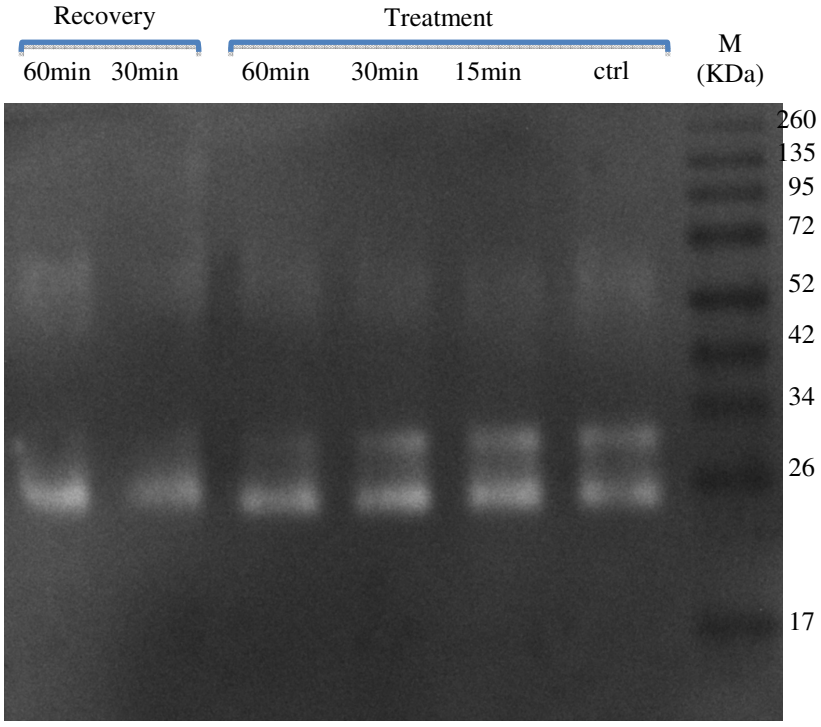


Fig.3. Effect of UVB treatment on D1 protein quantity at *Synechococcus sp.* PCC 7002.

We marked in the figure the periods when the samples were taken (ctrl-control, time 0, 15min, 30min and 60min) from the beginning of the treatment the samples took during the recovery period at 30 and 60 minutes.

The *Synechococcus sp.* PCC 7002 culture in the logarithmic growth phase (the chlorophyll concentration at 6µg/ml) was treated with UVB and samples were taken at 15, 30, 60 minutes. The control sample was taken at time 0. During the recovery time with optimal light intensity samples were taken at 30 and 60 minutes.

The samples had about 30µg chlorophyll/ml. They were centrifuged 5 minutes at 10000g and 4^0C, the pellet was saved and kept at -80^0C. From the frozen pellet we isolated proteins using a sonication protocol (Cockshut A., 2006).

From the immunoblott (fig.4) can be seen the treatment of the *Synechococcus sp.* PCC 7002 strain with UVB light had no effect on D1 protein expression, this protein is approximately at the same level in all samples.

3. Study of microaerobiosis effect on the photosystem II function.

The *Anabaena variabilis* ATCC29413; *Cyanothece sp.* ATCC51142 and *Synechococcus sp*. PCC7002 strains were grown by bubbling air 100%, then the microaerobiosis conditions were induced by bubbling 100%N_2. Then, samples were taken as follows : control bubbled with 100% air, 15 min, 30 min and 60 min respectively bubbled with 100% N_2, and recovery samples at 30min rcv and 60 min rcv bubbled with 100% air. We illustrate here the microaerobiosis influence on the *Synechococcus* sp.PCC 7002 strain.

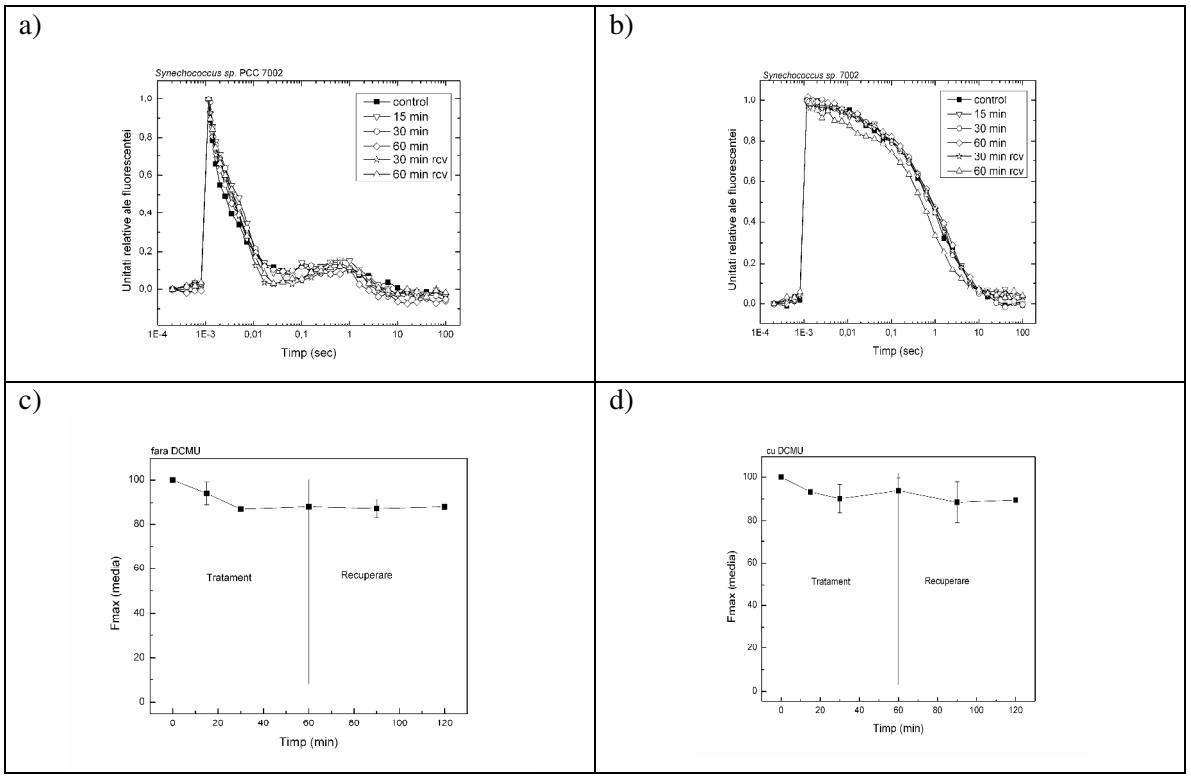


Figure 4. The microaerobiosis influence over the photosystem II function at *Synechococcus sp.* PCC7002

Fig.4 illustrates the microaerobiosis effect on the donor (panel a) and acceptor side (panel b) of photosystem II, by measuring the flash fluorescence curves in presence and absence of DCMU. It can be seen that the microaerobiosis treatment has not a notable effect over the electron transfer in the acceptor side of photosystem II (panel a), without having an effect on the donor side (panel b). In panels c and d is shown the N_2 treatment influence over the active centers of the photosystem II (panel c) and on the total number of photosystems II (panel d).

After the visit of our collaborators from Hungary, we made a number of circadian rhytm experiments at *Cyanothece* sp.ATCC51142 strain and we made also an advanced processing of fluorescence curves, by applying a Joliot correction over the normalized data, step followed by achieving a fitting to curves.

The Joliot correction is applied by the formula:

$Q_A^- (t) = D_1/[1-0.5*(1-D_1)]$

Where D1 indicates the first value from the normalized column and 0.5 is a constant value.

The curves Fitting is realized through the formula:

y = y0+A1*exp(-(x-x0)/T1)+A2*exp(-(x-x0)/T2)+A3/(1+(x-x0)/T3)

The function has 2 exponential components and one hyperbolic equation, so that a curve with Joliot correction and fitting will give us details over the time of each step from the fluorescence curves, and the standard errors applied for each step.

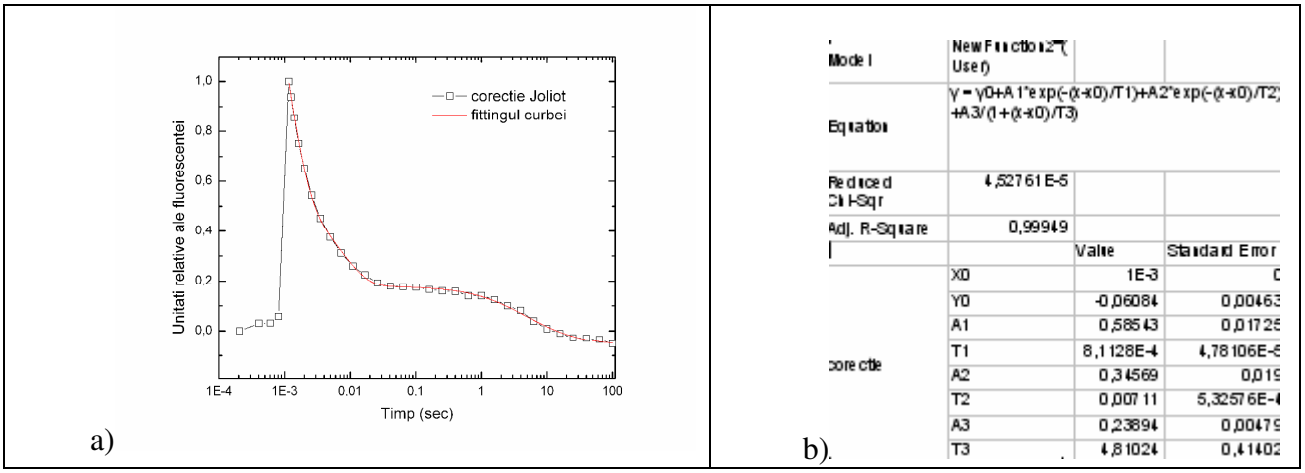


Figure 5. Applying the Joliot correction and the fitting to a fluorescence curve. In panel a) is illustrated the fluorescence curve measures (black square) and the curve obtained mathematically after the fitting (red continuous line). In panel b) are shown the equation parameters displayed by the fitting process.

Date: 3rd of December 2013

Project director,
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