INFLUENCE OF 100 GY GAMMA IRRADIATION ON THE GROWTH AND BIOCHEMICAL COMPOSITION IN *Synechocystis* PCC 6803 AND *Chlorella sorokiniana* UTEX 1230

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Abstract. The aim of this work was to characterize the response of cyanobacterium *Synechocystis* PCC 6803 and green microalgae *Chlorella sorokiniana* UTEX 1230 to 100 Gy gamma irradiation with respect to generation time and their content in valuable biochemical compounds. The ^{60}Co irradiation was carried out as previously described (MOISESCU et al., 2019) but the biological material (both the cyanobacterium and the microalga) was prepared in a slightly different way. Experimental results showed that in 100 Gy gamma irradiated *Synechocystis* PCC 6803 there are differences with respect to the growth rate, compared to the control. For *Chlorella sorokiniana* UTEX 1230, the experimental results showed that in 100 Gy gamma irradiated cells there are differences in the growth rate, total proteins, carotenes, total lipids and chlorophylls (i.e., chlorophyll *a* and *b* as well as biomass, when the cultures where scaled up from 50mL to 10 L). The results are discussed given the potential biotechnological applications, as well as from a fundamental scientific point of view.

Keywords: gamma radiation, *Synechocystis* PCC 6803, *Chlorella sorokiniana* UTEX 1230, generation time, lipids, total proteins, carotenoids.

Rezumat. Influența iradierii cu 100 Gy radiație gamma privind creșterea și compoziția biochimică în *Synechocystis* PCC 6803 și *Chlorella sorokiniana* UTEX 1230. Scopul acestei lucrări a fost caracterizarea răspunsului cyanobacteriei *Synechocystis* PCC 6803 și al microalgei verzi *Chlorella sorokiniana* UTEX 1230 la o iradiere gamma de 100 Gy, în ceea ce privește timpul de generație și al conținutului în compuși cu valoare biotehnologică. Iradierea gama folosind ca sursă ^{60}Co a fost efectuată așa cum s-a descris anterior (MOISESCU și colab., 2019), însă materialul biologic (atât cianobacteria cât și microalga) a fost preparat într-un mod ușor diferit. Rezultatele experimentale au arătat că în celulele de *Synechocystis* PCC 6803 iradiate cu 100 Gy există diferențe în ceea ce privește rata de creștere comparativ cu celulele control. În cazul *Chlorella sorokiniana* UTEX 1230, rezultatele experimentale au arătat că în celulele iradiate cu 100 Gy radiație gama, există diferențe în ceea ce privește rata de creștere, proteinele totale, carotenii, lipidele totale și clorofilele *a* și *b*, precum si biomasa totală atunci cand culturile sunt trecute de la 50mL la 10L. Rezultatele sunt discutate având în vedere potențialele aplicații biotehnologice, precum și din punct de vedere fundamental științific.

Cuvinte cheie: radiație gama, *Synechocystis* PCC 6803, *Chlorella sorokiniana* UTEX 1230, timp de generație, lipide, proteine totale, carotenoizi.

INTRODUCTION

The interaction between different types of microorganisms and gamma irradiation is a widely debated topic especially when it comes to high doses that inhibit cellular growth and multiplication, causing massive cell death (BRIDGES 1971; HANSEN & SHAFFER 2001; CHOI et al., 2014; LIU et al., 2015). Interestingly, the work at low doses receives less attention as compared with inhibitory doses, and up to our best knowledge there are only three reviews papers published on this topic (PLANEL et al., 1976; ARDELEAN et al., 2020a and b). However, there has been an increasing interest in using relatively low doses of gamma irradiation to stimulate biological processes in different types of microorganisms, including cyanobacteria (CONTER et al., 1986, 1987; HU et al., 1990; WANG et al., 1998; BADRI et al., 2015; MOUSSA et al., 2015; SHABAN et al., 2017) and microalgae (ERMAVITALINI et al., (2017a and b); GOMES et al., 2017; TALE et al., 2017; ABO-STATE et al., 2019; MOISESCU et al., 2019).

RIVASSEAU et al. (2010) reported that in a green Chlorophycean microalga grown under 450–2000 Gy irradiation, the pools of some free amino acids increased in the cell, as compared with the control. TALE et al. (2017) used gamma irradiation at dose rate of 3.097 kGy h^{-1} as a stressor to induce lipid hyper-accumulation (up to 40% of biomass) in *C. sorokiniana* KMN2 and *C. sorokiniana* KMN3, , reacher in shorter carbon chain fatty acid (i.e., C-16) compared to longer chain fatty acids. JEONG et al. (2017) have shown that chronic LDR-type irradiation induces increased cell densities, specific growth rates, and biomass of *T. suecica, D. tertiolecta* and *P. tricornutum* where there is also an increase in the amount of lipids, with a maximum in *T. suecica* when irradiated with a dose rate of 6 mGyh-1.

ERMAVITALINI et al. (2017a) showed that *Botryococcus* sp. irradiated at very low doses (2, 4, 6, 8, and 10 Gy) changes the characteristics of their growth, biomass, percentage of total cell lipids and fatty acid profile. More precisely, the highest biomass (0.833 g) and lipid content (41 % total biomass) were found in the 10 Gy irradiated microalgae. Later, ERMAVITALINI et al. (2017b) analysed the fatty acid profile of *Botryococcus* sp. control cells and found only 6 types of fatty acids while in 10Gy irradiated microalgae cells found 12 types of fatty acids, with an increased proportion of long chain fatty acids and a low proportion of short chain fatty acids. MOISESCU et al. (2019) demonstrated that the generation time of *Chlorella sorokiniana* UTEX 1230 decreases to 56% at 10 Gy, 60% at 50 Gy, and 77% at 100 Gy irradiation and the relative lipid content increases by 20% and 50% after 10 Gy and 100 Gy irradiation, respectively.

In the *Synechococcus lividus* CONTER et al. (1986) have shown that very low chronic doses of γ-radiation (53.5 mGy/year) stimulate proliferation of the cells, induced a high superoxide dismutase (SOD) activity followed by concomitant peaks of glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6P-DH). Furthermore, there is an increase in the pigment content and an enhancement of glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) and the degradation of phycocyanin, thus demonstrating that cells were submitted to a photooxidative stress. Later on, CONTER et al. (1987) also demonstrated that chronic γ-irradiation with doses ranging from 0.058 mGy/ day to 0.204 mGy/day had a stimulatory effect on nucleic acid synthesis and induced an increase in SOD, GR and G6P-DH as a response to the oxidative stress. HU et al. (1990) reported that low doses of γ-rays, less than 1 kGy, could stimulate the growth of *A. platensis*. Small changes in the morphology of the filament were found at doses less than 0.5 kGy.

WANG et al. (1998) studied the effect of γ-radiation (up to 6 kGy) on the growth and morphology of four different strains of *Arthrospira* sp. and concluded that it showed resistance to γ-irradiation with stimulation of growth at low doses, while the filaments would break up or even disintegrate at high doses. RAZI & HASNAIN (2006), studied the effect of γ-rays on the growth parameters of two chromium resistant unicellular cyanobacteria, from the genus *Synechocystis* sp. Both strains showed a significant increase in chlorophyll content when irradiated at doses of 1 to 10 Gy. Carotenoid content increased significantly only in strain AHZ-HB-MK (DQ381960) sp. at all growth stages. MOUSSA et al. (2015), exposed *A. platensis* to different γ-radiation doses (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy) and found that the optimum upsurge for chlorophyll a, carotenoids, intensity of total photosynthetic activity, and carbohydrates is at 2.0 kGy. Ribuloso-1,5-bisphosphate carboxylase / oxygenase (RUBISCO) activity peaked at 2.0 kGy and phosphoenol-pyruvate-carboxylase activity (PEPCASE) peaked at 1.0 kGy. Another important study, (BADRI et al., 2015) showed that *Arthrospira* sp. PCC 8005 is highly tolerant to γ-rays and can survive to at least 6400 Gy (dose rate of 527 Gy/h). Their detailed proteomic and transcriptomic analyses performed after irradiation with 3200 or 5000 Gy showed a decline in photosystem II quantum yield, reduced carbon fixation, and reduced pigment, lipid, and secondary metabolite synthesis. On the other hand, transcription of photo-sensing and signalling pathways, and thiol-based antioxidant systems was induced. Furthermore, transcriptomics did show significant activation of ssDNA repair systems and mobile genetic elements (MGEs) at the RNA level. Interestingly, the cells did not induce the classical antioxidant or DNA repair systems, such as superoxide dismutase (SOD) enzyme and the RecA protein.

Arthrospira sp. cells lack the catalase gene and the LexA repressor. Based on the observation that irradiated *Arthrospira* cells did induced strongly a group of conserved proteins, the authors (BADRI et al., 2015) put forward the hypothesis that these proteins could be involved in the response of cyanobacterial cells to irradiation, which remains to be checked. ABOMOHRA et al. (2016) showed that in the cyanobacterium *Arthrospira platensis* carbohydrate production by 106, 246 and 146%, respectively and lipid content increased significantly over the control at 0.5 kGy. Interestingly, carotenoid productivity showed significant increase at all used γ-rays doses up to 155% over the control whereas other components decreased. SHABANA et al. (2017) showed that in *A. platensis* exposed to doses up to 2.0 kGy, significantly increased the phenolic and proline contents and stimulated the soluble proteins, malondialdehyde (MDA), vitamins (A, K and B group) and mineral (N, P, Na, K, Ca, Mg and Fe) contents.

The activities of some N-assimilating and antioxidant enzymes were significantly increased at irradiation doses up to 2.0 kGy. This study withstands the possible use of γ-irradiation as a stimulatory agent to raise the nutritive value and antioxidant activity of *A. platensis*. Gamma rays ionize water and generate ROS (reactive oxygen species, which include hydrogen peroxide, H2O2, superoxide, O2 – , and hydroxyl radicals, HO–) which interact with intracellular macromolecules, causing damages and activating cellular pathways for repairing them (ROBINSON et al., 2011). The higher the dose of γ-radiation penetrates the cell, the greater the amplitude of damages and the complexity of repairing mechanisms, including DNA-repairing mechanisms, inorganic scavengers (e.g. salts and Mn++ ions) and organic scavengers (carotenoids and ROS scavenging enzymes). ROS and ROS-altered cellular compunds could interact with members of signaling pathways to induce changes in some gene expression, as suggested by TALE et al. (2017), for upward regulation of lipid biosynthetic pathway, and by BADRI et al. (2015), for a group of conserved proteins. We expect that the γ-irradiation at low doses could increase synthesis of some compounds important for cell biology and for biotechnological and medical applications.

Following our previous work on stimulating lipid production by photosynthetic microorganisms through natural strain selection (ARDELEAN et al., 2017), random chemical mutagenesis (ARDELEAN et al., 2018) and gamma irradiation at different doses (MOISESCU et al., 2019; ARDELEAN et al., 2020 a, c), in this paper we present the results obtained on the cyanobacterium *Synechocystis* PCC 6803 and green microalga *Chlorella sorokiniana* UTEX 1230, irradiated with 100Gy, with special emphasis on the results concerning the scaling up of green microalga cultures from 50 mL to 10 L.

MATERIALS AND METHODS

The photosynthetic microorganisms used in this paper are the cyanobacterium *Synechocystis* PCC 6803 and the green algae *Chlorella sorokiniana* UTEX 1230.

The protocols for the determination of the lipid content, chlorophyll *a* and *b*, total carotenoids and total soluble proteins are as previously shown (ARDELEAN et al., 2020b).

In short, the **generation time** was determined based on optical density readings, using the software accessible at the link www.doubling-time.com/compute.php. It was performed for 3 growth cycles, with 7 days each.

Lipid content was estimated by the phosphor vanillin method (PARK et al., 2016). Over the dry sample with a known amount of biomass, 0.1 mL of concentrated sulphuric acid was added and then heated at 90°C for 10 minutes. After that, the samples are cooling and 2.4 mL of phospho-vanillin reagent are added in each sample. Then, the samples are placed at 37 C in a shaking incubator for 15 minutes and the colour of the sample turned pink. The optical density of the samples is read at 530 nm.

The equation for the standard curve is as follows: $y = 0.0034x-0.006$ and $R^2 = 0.9826$.

Chlorophyll *a* and *b* was extracted in 90% methanol and the concentration was calculated as previously shown (ARDELEAN et al., 2018). [Chl a+ Chl b] = 22.12 E652.0 + 2.71 E665.2 (PORRA, 2002)

Total carotenoids were measured spectrophotometrically using the modified method of MACKINNEY (1941) as presented by (BOYER, 2006). Carotenoids were estimated using the following equation: Carotenoids (µg/ mL) = 4.2 A452 – [0.0246 (10.3 A665 – 0.918 A650)].

Total soluble proteins were estimated using the biuret method. After carotenoid extraction, residual cells were extracted using 1 N NaOH in a boiling water bath for 2 h. The equation for the standard curve is as follows: $y = 0.0346x-0.0004$ and $R^2 = 0.9998$.

Gamma irradiation. Acute gamma irradiations (0.9 Gy/s) were performed by using a research, self-contained Co-60 gamma irradiator (GC-5000 – B.R.I.T., India). The dose was 100 Gy. The dose uniformity ratio (D.U.R.), defined as the ratio of maximum dose to minimum dose in the samples, was 1.259 (+/- 4.7 %). Doses were evaluated by means of ethanol-chloro-benzene (ECB) dosimetry system with oscillometric read-out (ISO/ASTM 51538). Doses are expressed as absorbed dose in water and their corresponding uncertainty is 3.3 %.

For scaling up the irradiated culture at 10L, gamma irradiation was applied as previously shown (MOISESCU et al., 2019; ARDELEAN et al., 2020a) with the exception that 12 tubes (50mL algal culture each) were irradiated instead of 6, as in previous experiments. Other 12 tubes (50mL algal culture each) were kept as control.

RESULTS AND DISSCUSIONS

Generation time

Table 1 presents the generation time for the green microalga *Chlorella sorokiniana* UTEX 1230 irradiated with 100Gy. These results obtained on eukaryotic photosynthetic microorganisms are promising with respect to the aim of this paper, especially in the case of *Chlorella sorokiniana* UTEX 1230.

Table 1. Generation time for *Chlorella sorokiniana* UTEX 1230 following irradiation with 100 Gy, during 3 growth cycles, 7 days each cycle.

Growth cycle	Generation time (h)	% change
1	72.115	100
2	141.54	196.27
3	122.66	170.09

In Table 2 there are presented the generation time for the cyanobacteria *Synechocystis* PCC 6803 irradiated with 100Gy.

Table 2. Generation time for *Synechocystis* PCC 6803 following irradiation with 100 Gy, during 3 growth cycles, 7 days each cycle.

Growth cycle	Generation time (h)	% change
1	51.40	100
2	75.90	147.67
3	71.16	138.43

In tables 1 and 2, the generation time in the first growth cycle is taken as 100% and growth cycles 2 and 3 are reported to the first.

For both strains, *Synechocystis* PCC 6803 and *Chlorella sorokiniana* UTEX 1230, one can see that after gamma irradiation the first growth cycle corresponds to a shorter generation time (72 h for *Chlorella sorokiniana* and 51 h for *Synechocystis)*, as compared with the following growth cycles (141 and 122 h for *Chlorella* , respectively 75 and 71 h for *Synechocystis)* . A shorter generation time means that the time between two successive divisions is shorter, so the population doubles faster in these conditions. This result suggests that the stimulatory effect of 100 Gy gamma irradiation took place for the first 7days after irradiation, then stopped.

Figure 1 shows an image of *Chlorella sorokiniana* UTEX 1230 cultures scaled up at 10L (control and 100 Gy irradiated cells) at the end of the first growing period (7 days) .The samples were mixed with a pump during this period, kept in the incubator at 20 °C and continuous white fluorescent light.

Figure 1. *Chlorella sorokiniana* UTEX 1230 cultures irradiated at 100Gy and the control scaled up at 10L after 7 days of growth.

Figure 2 shows the concentrations of lipids, total proteins and total carotenes as well as chlorophylls *a* and *b*, in cells irradiated with 100 Gy as well as in non-irradiated cells (control). It can be seen that, as expected based on the previous results, the concentrations of these compounds are higher in the irradiated cells than in the control.

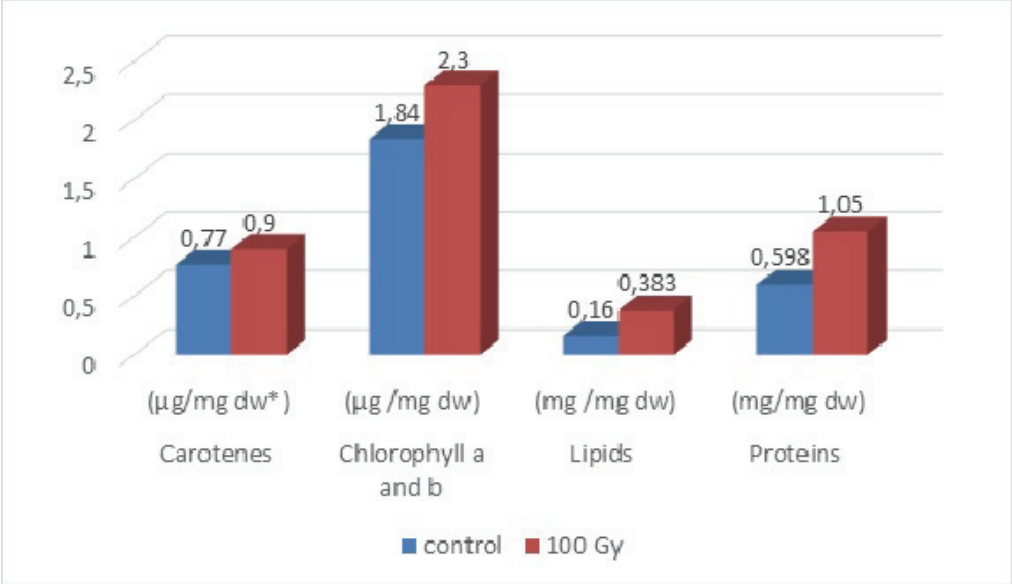

Figure 2. Chemical composition of *Chlorella sorokiniana* UTEX 1230 grown in 10 L bioreactors (control and 100 Gy irradiated cultures(*dw= dry weight).

One can see that 100 Gy gamma irradiated cells contain higher concentrations of carotenes, chlorophylls, lipids and proteins which sustain the main results previously obtained at this irradiation, at the level of 50mL cultures.

Table 3 presents the calculations concerning the quantity of the above substances, taking into account the biomass accumulated during 7 days of growth for each sample, control and irradiated.

Table 3. Total dry weight of biomass, lipids, proteins, chlorophylls a and b, and total carotenes in *Chlorella sorokiniana* UTEX 1230 control and 100 Gy irradiated

	Total dry biomass in 10L (mg dw)	**Total lipids (mg)**	**Total proteins (mg)**	**Total chlorophylls *a* and *b* (µg)**	**Total carotenes (µg)**
Control	1250	200	747.5	2300	962.5
100Gy	725	277.67	761.25	1667.5	652.5

As one can see in table 3 the biomass concentration is lower in the irradiated cells as compared with the control. This is not only an unexpected result, but un unpleasant one, with respect to the biotechnological potential of low gamma irradiation as a stressor to stimulate the cell growth. At this time, we do not have an explanation for this unexpected result, it could be linked to the growth conditions, which are different at 10L level than in 50mL Erlenmeyer flasks. As long as the mechanisms by which gamma irradiation influences the synthesis of given chemicals is unknown it is difficult to find a correct explanation for these results.

There are experimental evidence indicating that reactive oxygen species (ROS) may be important mediators in lipid accumulation by different types of microorganisms, including microalgae (YILANCIOGLU et al., 2014; SHI et al., 2017; TALE et al., 2017). TALE et al. (2017) showed that the exposure to gamma ray is able to generate ROS, including OH• and H_2O_2which can affect cellular morphology, biochemistry, and physiology in photosynthetic cells.. Their results showed that in response to gamma irradiation, there is an instant and huge build-up of ROS inside microalgal cells. It was showed that the proper exposure to exogenous added ROS can trigger neutral lipid formation without any other separate or distinct stress (YU et al., 2015). For example, it was shown by BATTAH et al. (2014) that after 8 days of treating *C. vulgaris* with 2 mM and 4 mM H_2O_2 the lipid content increased by 20 % and 87 %, respectively compared to the control (BATTAH et al., 2014). These observations indicated that oxidative stress is accompanied by an increased lipid content in the green alga. In addition, they showed that at optimum cultivation conditions, inducing oxidative stress by application of exogenous H_2O_2 leads to increased cellular lipid content up to 44% when compared with non-treated control groups, arguing that oxidative stress and lipid overproduction are linked.

The progress in knowledge would help us to understand the processes involved in affecting the growth and chemical composition of microalgae following non-growth inhibitory treatments with gamma irradiation, and to improve biotechnological applications.

CONCLUSIONS

In agreement with our previous results, gamma irradiation with 100 Gy stimulates the growth of both photosynthetic microorganisms, the cyanobacterium *Synechocystis* PCC 6803 and the green microalga *Chlorella sorokiniana* UTEX 1230, when cultivated in 50mL volume.

The concentration of analysed chemicals is higher in 100 Gy irradiated cultures than in non-irradiated controls.

However, when scaling up from 50mL to 10L, the accumulated algal biomass is higher in control than in 100 Gy irradiated cultures, an unexpected result, which negatively affects the quantity of the desired chemicals.

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