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EFFECT OF ACUTE GAMMA IRRADIATION ON GENERATION TIME, LIPID, CHLROPHYLL *A* AND CAROTENS, IN *Chlorella sorokiniana* UTEX 2130 AND *Synechocystis* PCC 6803

Cristina MOISESCU[1], Ana-Valentina ARDELEAN[1, 2], Daniel NEGUT[3], Ioan I. ARDELEAN[1]

[1]Institute of Biology Bucharest, Romanian Academy, Splaiul Independenţei 296, District 5, 060031, Bucharest, Romania
[2]USAMV Bucharest, Faculty of Biotechnologies, 59 Mărăşti Blvd., District 1, 011464, Bucharest, Romania
[3] Horia Hulubei National Institute of Physics and Nuclear Engineering, IRASM Radiation Processing Department, 30 Reactorului Street, 077125, Măgurele-Ilfov, Romania

Corresponding author email: ioan.ardelean@ibiol.ro

Abstract

Microalgae are microorganisms very important for fluxes of matter, energy and information on planet Earth as well as for a plethora of biotechnological applications. In this paper, we present our original results concerning the use of acute gamma irradiation (0.9 Gy/s) to challenge the cells of Chlorella sorokiniana UTEX 2130 and Synechocystis PCC 6803.The main results obtained on Chlorella sorokiniana UTEX 2130 are: a) a great decrease in the generation time to 56% after 10 Gy irradiation, to 60% after 50 Gy irradiation, and to77% after 100 Gy irradiation, whereas b)the relative lipid content increased by 20% and 50% after 10 Gy and 100 Gy (as compared with the non-irradiated control). The main results obtained on Synechocystis PCC 6803 are: a) the generation time decreased to 90% after 10 Gy irradiation, to 85% after 50 Gy irradiation, whereas b) there is an increase in the chlorophyll a content (by 33%) and carotene (by 22%) after an irradiation of 50 Gy.

Key words*: acute gamma irradiation, lipids, carotenoids, Chlorella sorokiniana, Synechocystis PCC 6803.*

INTRODUCTION

Photosynthetic microorganisms, both prokaryotes and eukaryotes, have the ability to use water, carbon dioxide and some minerals to perform complex endergonic biochemical reactions enabling them to synthesize a plethora of cellular constituents thus sustaining their multiplication. Furthermore, some of them are capable of mixotrophic growth, using both inorganic and some organic substances as primary carbon sources. This is why photosynthetic microorganisms, in addition to the major planetary role in solar energy conversion and biogeochemical cycles, have a major contribution to a wide range of biotechnological applications (Whitton, 2012 and references herein). One such direction concerns the ability of the majority of microalgae to accumulate lipids inside the cell (Sheehan et al., 1998; Chisti, 2007; Li et al., 2008; Liang et al., 2009; Demirbas, 2010; Huang, 2010; Mata et al., 2010; Amaro et al., 2011; Schuhmann et al., 2012; Borowitzka, 2013; Rawat et al., 2013; Velea et al., 2014; Ardelean & Manea, 2016; Ardelean et al., 2017; 2018).

One important task is to increase the content of lipid inclusions of the strains in order to develop an economically viable biotechnology. In this respect, the main strategies are: i) the selection from naturally occurring strains of those with high lipid content; ii) the mutagenesis of this naturally occurring populations in order to further select the most productive cells; iii) the use of genetic engineering tools (including site directed mutagenesis and metabolic engineering) to change the metabolic flux toward lipid deposition, and iv) the control of growing conditions (the so called stressors) such as nutrient depletion, nitrogen starvation, pH, and temperature shock. All these strategies have the potential to increase the lipid content (% mass/mass) in microalgae (Sheehan et al.,

1998; Sharma et al., 2012; Sibi et al., 2016; Yong et al., 2019). Recently, a new stressor, namely Co60 γ-irradiation at low, non-lethal doses, received attention with respect to the induction of increased lipid content in irradiated microalgae (Tale et al., 2018; Ermavitalini et al., 2017).

The aim of this contribution is to challenge the cyanobacterium *Synechocystis* PCC 6803 and the green alga *Chlorella sorokiniana* UTEX 2130 by acute, non-lethal gamma irradiation in order to check if any changes in their phenotypic traits, with special emphasis on the generation time (in both strains) and lipid inclusions (only in the green alga) will occur.

RESULTS AND DISCUSSIONS

Doubling time

In Table 1 are presented the results concerning the calculated generation time (GT) for the cyanobacterium *Synechocystis* PCC 6803 irradiated at 10Gy, 50Gy, and 100Gy, as compared with the non-irradiated control.

Table 1. Generation time for *Synechocystis* PCC 6803 at different irradiation doses

Irradiation dose (Gy)	Generation time (h)	%
Control	77.09	100.00
10	68.93	89.41
50	65.26	84.65
100	81.29	105.45

The GT in control is taken as 100% and all other results are reported to the control. One can see that the GT is shorter in populations irradiated at 10Gy and 50Gy, as compared with the control, whereas the irradiation at 100Gy showed a longer GT, within the range of standard deviation (5%). The irradiation at 500Gy and 1000Gy indicated the cell death (results non shown).

In Table 2 there are presented the GT for the green alga *Chlorella sorokiniana* UTEX 2130 irradiated at 10Gy, 50Gy, and 100Gy, as compared with the non-irradiated control. The results showed that the GT is much shorter in populations irradiated at 10Gy, 50Gy, and 100Gy as compared with the control. The irradiation at 500Gy and 1000Gy induced the cell death, as in the case of cyanobacterium *Synechocystis* PCC 6803.

These results obtained on both prokaryotic and eukaryotic photosynthetic microorganisms are promising with respect to the aim of our goals, especially in the case of *Chlorella sorokiniana* UTEX 2130 where differences in GT reached values up to 30-40% in irradiated populations as compared with the control. However, at this time, there is no causal explanation for these differences and further studies are necessary in order to elaborate a true working hypothesis.

Table 2. Generation time for *Chlorella sorokiniana* UTEX 2130 at different irradiation doses

Irradiation dose (Gy)	Generation time (h)	%
Control	188.59	100.00
10	105.34	55.86
50	118.89	63.04
100	145.01	76.89

Lipid inclusions content

In Table 3, is presented the estimated lipid inclusions content of *Chlorella sorokiniana* UTEX 2130 population irradiated at 10 Gy and 100 Gy, as compared with non-irradiated control. The estimation of lipid inclusions content by Nile red fluorescence method showed a much stronger increase in the fluorescence signal after Nile red addition in irradiated populations (10 Gy and 100 Gy) as compared with the control.

Table 3. The intensity of the fluorescence signal without (-) and with (+) Nile red and the calculated ratio (+/-) and % difference

Irradiation dose (Gy)	(-) Nile Red	(+)Nile Red	Numerical ratio (+/-)	%
Control	2222	9402	4.23	100.00
10	1594	8152	5.11	120.80
100	1823	11655	6.39	151.06

These results are very promising because the increased lipid droplet content (20% and 50% in the 10Gy and 100Gy irradiated cells, occurs in populations with shorter GT than in the control (55.86% and 76.89%, respectively). Interestingly, the increase in lipid droplet content occurs when cells are grown in normal conditions, without any attempt (so far) to manipulate the growing conditions in order to facilitate intracellular deposition of lipid droplet, such as the decrease of nitrogen concentration (Yong et al., 2019).

In Figure 1 there are presented individual cells of *Chlorella sorokiniana UTEX* 2130 after

labelling with Nile red, irradiated populations as compared with the control. There are presented only images obtained in green fluorescence,

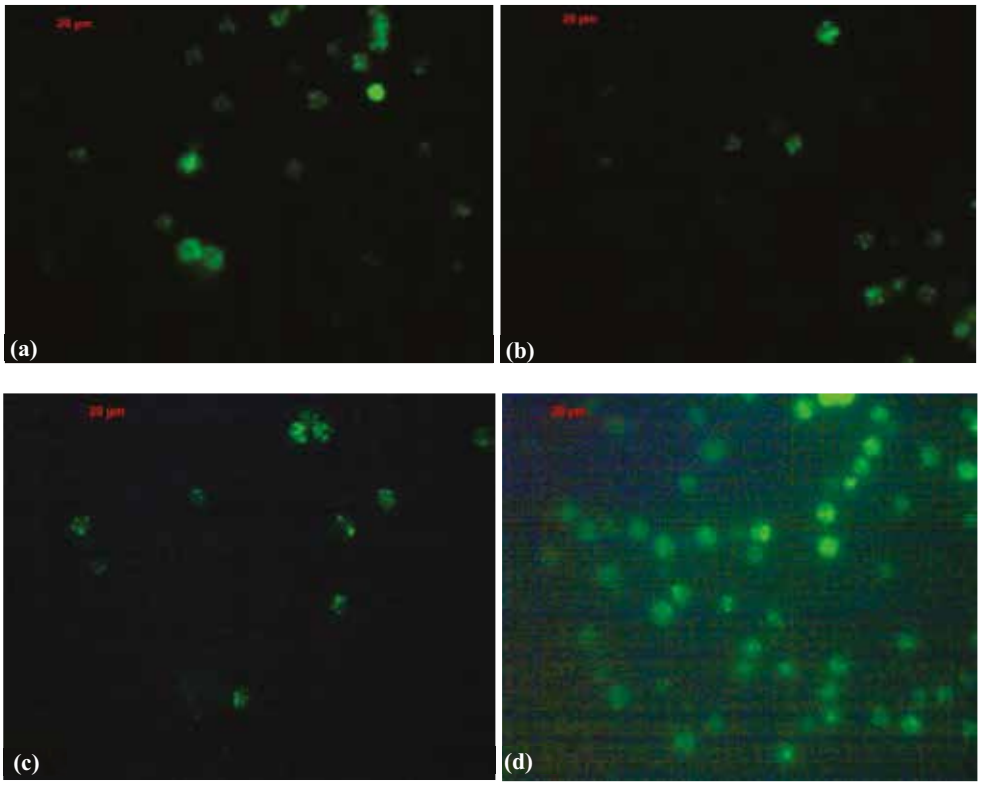

Figure 1. Individual cells of *Chlorella sorokiniana* UTEX 2130 after labelling with Nile red: (a) control, non-irradiated cells; (b) 10Gy irradiated cells; (c) 50Gy irradiated cells; (d) 100Gy irradiated cells

where only the emission of Nile red in the hydrophobic ambient of lipid droplets is evident (Greenspan, 1985) and one can see an increased surface (corresponding to labelled lipid droplets) emitting green fluorescence. The images in red fluorescence are not shown as the red fluorescence of Nile red lipid droplets is mixed together with the red fluorescence of chlorophylls in the alga.

Microscopic investigations show larger areas inside the cells and also a larger number of cells emitting green fluorescence, which indicates that these cells contain a higher lipid inclusions content. These microscopic images only document the presence of lipid droplets without any numerical quantification, as no image analysis has yet been performed on them. The lipid inclusions content was not estimated in the cyanobacterium *Synechocystis* PCC 6803 as wild cyanobacterial strains do not have true lipid inclusions, besides polyhydroxybutyrate which is a common inclusion in cyanobacteria.

Chlorophyll *a* and carotenoids content in *Synechocystis* PCC 6803

In the next table one can see the concentration of chlorophyll *a* and carotenoid pigments in *Synechocystis* PCC 6803.

Table 4. The concentration of chlorophyll *a* and carotenoid pigments in *Synechocystis* PCC 6803

Irradiation dose (Gy)	Chlorophyll *a* (µg/mL)	Carotenoid pigments (µg/mL)
Control	12.65	8.20
10	14.48	9.37
50	16.77	10.05
100	14.71	9.76

The results in the above table show that there are some changes in the chlorophyll *a* and carotenes content. Taking the control as 100% the increase in chlorophyll *a* concentration went up to 114, 132, and 116%, respectively at 10, 50, and 100Gy. The same increases were registered for the total carotenes (i.e. 114, 122, and 119%).

The cyanobacterium *Synechocystis* PCC 6803 and the green alga *Chlorella sorokiniana* UTEX 2130 had been chosen for our experiments as they are versatile photosynthetic microorganisms intensively used in both fundamental and applicative research (Whitton, 2012; Lizzul et al., 2018 and references herein) able to grow either strictly photosynthetically but also mixotrophic or even heterotrophic, not only on pure organic substances but also on waste feedstock, with short doubling times. The scientific community strongly agree that these strains have a strong industrial potential (Lizzul et al., 2018; Whitton, 2012 and references herein; Hu et al., 1990) studied the effect of gamma radiation on the growth and morphology of *A. platensis*. They reported that low doses of gamma rays, less than 1 kGy, could stimulate its growth. Small changes in the morphology of the filament were found at doses less than 0.5 kGy. The LD_{50} was 1.0 kGy, while 2.5 kGy caused 100% lethality. Wang et al. (1998) studied the effect of gamma radiation (up to 6 kGy) on the growth and morphology of four different strains of *Arthrospira* sp. and concluded that it showed resistance to gamma irradiation with stimulation of growth at low doses, while the filaments would break up or even disintegrate at high doses. Although many studies have evaluated the biological response of microalgae to high doses of gamma radiation, few studies have focused on stimulation of bioactive compounds production in *A. platensis.*

Abomohra et al. (2016) studied the influence of gamma radiation on the growth and production of some active substances of the cyanobacterium *Arthrospira platensis*. In their important paper they found the following: i) biomass production was significantly inhibited by 21 and 34%, with respect to the control at 2.0 and 2.5 kGy, respectively and chlorophyll *a* content showed 11% reduction at 2.5 kGy compared to the control. In addition, phycobiliproteins productivity showed a significant decrease by 10 and 36% below the control at 2 and 2.5 kGy of gamma radiation, respectively whereas protein production was decreased significantly with the concomitant increased of 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 gamma doses up to 155% over the control.

In a recent paper (Ermavitalini et al., 2017), the work done on *Botryococcus* sp. irradiated at low doses (2, 4, 6, 8 and 10 Gy) showed that irradiation using gamma rays changed the characteristics of their growth, biomass, percentage of total lipids cell and fatty acid profile. More precisely, the highest biomass and lipid content found in 10 Gy irradiated microalgae are 0.833 g biomass and 41 % lipid content. Furthermore, fatty acid profile of *Botryococcus* sp. control has 6 fatty acids while 10 Gy irradiated microalgae has 12 fatty acids, with the long-chain fatty acids increased, whereas short-chain fatty acids decreased. Tale et al. (2018) used gamma irradiation as a stressor to trigger lipid accumulation in two strains of *Chlorella sorokiniana* (i.e. *C. sorokiniana* KMN2 and *C. sorokiniana* KMN3). These strains were treated by Co60 γ-irradiation, in the range of 100 to 1100 Gy, much higher than the dosses used by us in the algal strain *Chlorella sorokiniana* UTEX 2130. The authors (Tale et al., 2018) reported the enhancement of the lipid content of more than 40% of biomass as well as the level of intracellular reactive oxygen species. They also showed that the expression patterns of regulatory genes involved in lipid biosynthesis, namely acetyl-CoA carboxylase and diacylglycerolacyl transferase, were up regulated immediately after irradiation and were highest 3 days post irradiation (Tale et al., 2017). The authors also performed the analysis of the fatty acids composition in irradiated and control populations, showing that γ-irradiation induced lipid enhancement is accompanied by higher accumulation of shorter carbon chain fatty acid (C-16) compared to longer chain fatty acids. They claimed that the novel strategy of using gamma radiation as a faster andextremely potent stressor for triggering lipid biosynthesis

in microalgae has immense potential in industrial biodiesel production. They put forward the hypothesis that γ-irradiation in microalgae causes oxidative stress and up-regulationof lipid biosynthetic pathway which in turn leads to lipid accumulation.

Another study (Badri et al., 2015) showed that *Arthrospira* sp. PCC 8005 is highly tolerant to gamma raysand 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 5000Gy 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.

In our opinion, the interactions between photosynthetic microorganisms (both prokaryotes and eukaryotes) and gamma irradiation, at low, non-mutagenic doses, is still in its infancy.

CONCLUSIONS

Acute gamma irradiation in *Chlorella sorokiniana* UTEX 2130 showed the followings: a) a great decrease in the GT to 56% after 10Gy irradiation, to 60% after 50Gy irradiation, and to7 7% after 100 Gy irradiation, whereas b)the relative lipid content increased by 20% and 50% after 10Gy and 100Gy as compared with the non-irradiated control.

Acute gamma irradiation in *Synechocystis* PCC 6803 show that: a) the GT decreased to 90% after 10Gy irradiation and to 85% after 50Gy irradiation, whereas b) there is an increase in the chlorophyll *a* content (by 33%) and carotene (by 22%) after an irradiation of 50 Gy.

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