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Biochemical composition and antioxidant potentiality of Synechococcus mundulus and Arthrospira platensis in response to ionizing radiation

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Abstract: This study aimed to investigate the effect of ionizing gamma irradiation on variation in the biochemical composition and change in the antioxidant activity of Synechococcus mundulus and Arthrospira platensis. Cultures of S. mundulus and A. platensis were irradiated with two doses of gamma irradiation (1.0 and 2.0) kGy. Both irradiated and control cultures were allowed to grow in BG11 medium for 21 and 24-d for S. mundulus and A. platensis, respectively. In the irradiated cultures, biochemical analysis revealed an increment in the total carbohydrate content in both A. platensis and S. mundulus, meanwhile total free amino acids recorded significant increment in A. platensis and non-significant in S. mundulus. Though soluble protein content was significantly decreased in irradiated cultures of A. platensis and non-significant decrease in S. mundulus. In addition, super oxide dismutase potentiality was also significantly activated in the irradiated cultures.

keywords: : Arthrospira platensis, Antioxidant potentiality, Biochemical composition, Ionizing radiation, Synechococcus mundulus.

1.Introduction

Cyanoprokaryota are primitive, prokaryotic, photosynthetic microorganisms, form a heterogeneous collection of oxygen-evolving microbes which could be appeared during the Precambrian age and produced the environmental oxygen which maintained the present life [1]. Cyanobacteria occupy majority of the natural illuminated ecosystem both aquatic (freshwater or marine) and terrestrial, as well as various types of severe environments such as hot springs, Arctic and Antarctic lakes and rely on sun energy to perform their metabolic process via harvesting solar energy [1, 2]. As a result of cyanobacteria genetic capability to grow in various environmental stresses, they have promoted distinguishable adaptability and developed effective protective strategies to face these changes in the environmental conditions [3]. Cyanobacterial species populate a wide range of ecosystems such as marine and freshwater, cold and hot environments, alkaline and acidic, and symbiotic associations [4]. The wide dispersion of cyanobacterial species also plays a major role in physiological characteristics and resistance to different environmental factors [5]. Cyanobacterial eco-physiological adaptation appears clearly in tolerance to different environmental factors such as UV-irradiation by developing many strategies, for example, over secretion of UV─ protective metabolites including scytonemin, mycosporine-like amino acids and carotenoids, developing enzymatic defense mechanisms like super oxide dismutase (SOD) and repair UV damaged proteins [6]. *Arthrospira platensis*, also known as *Spirulina*, is a non-toxic, planktonic free floating cyanobacterium, has cylindrical, filamentous shape and multicellular system with size ranges from 0.2 to 0.3 mm and flourish in both tropical and subtropical areas [7, 8]. *Arthrospira spp.* has a fast growing capability; it develops vigorously in high carbonate and bicarbonate medium with

alkaline pH quantified to 9.5-11 that inhibit growth of other cyanobacterial species [8, 9]. *Arthrospira* spp. acquires great interest as health enhancing food supplement due to their fast growing, simple harvesting and processing, benign nature, high protein content, vital fatty acids, and essential minerals and vitamins [10, 11].

Picocyanobacteria is a sub-group of cyanobacteria which includes two genera: *Prochlorococcus* and *Synechococcus*, and characterized by their small sized single cell (less than 2 µm) [12]. Their dispersal in aquatic regions has been studied, indicating that members of *Synechococcus* prefers mesotrophic environments, and are abundant in freshwater meanwhile, *Prochlorococcus* spp. are very plentiful in oligotrophic marine regions with concentrations of 10^6 and 10^5 cells / ml, respectively [12-14]. *Synechococcus* is a polyphyletic group due to its ubiquitous nature whereas it colonizes both fresh and sea water Marine *Synechococcus* spp. inhabit the upper illuminated layer of coastal, estuarine and offshore areas, as well as wide range of temperatures and nutrients [15, 16]. Picocyanobacteria faces enormous variations in irradiance as a result of combination between light and dark, water movement and cloudiness percent so it develops a different defense mechanisms such as thermal elimination of additional light, as well as removing reactive oxygen species via structural modification of the photosynthetic machinery to ensure their survival in excess illumination [17, 18]. Effects of gamma irradiation (γ-irradiation) on growth, physiological activities and genomic material of cyanobacterial and algal cells have been studied [19, 20]. Damage of nucleic acids, proteins and was proposed to be the main targeting cell components by gamma irradiation in biological cells, meanwhile by the mid-1960s, most studies have focused on cell death by DNA damage [21]. In the last decades, scientist attempted to interpret the mechanism by which radio-resistant organism can sustain with gamma irradiation.

The current study aimed to investigate the growth responses of two cyanobacterial species against acute γ-irradiation doses, and quantifying variation in the total carbohydrate, total soluble protein, total amino acids and SOD activity at stationary phase after irradiation.

2. Materials and methods

Organisms isolation and cultivation conditions

Arthrospira platensis and *S. mundulus* were provided by the algal collection of the phycology lab (Faculty of Science, Mansoura University) and cultured using BG11 nutrient medium [22]. The cultures were activated under low illumination (34.5 µmol photons m-2 s-1) in a photo cycle consisting of 16 h light and 8 h dark and incubated for 21 d (late stationary growth phase) at 29±2 °C.

2.2 Exposure to γ-irradiation and estimation of biochemical components:

Cultures samples (100 ml) of *A. platensis* or *S. mundulus* were irradiated in dark gamma cell about at 25±2 with two acute doses (1.0 and 2.0 kGy) of gamma rays using Co^{60} as gamma rays source at the Cyclotron unit, Nuclear Research Center, Egyptian Atomic Energy Authority (EAEA) - Egypt, with dose rate quantified as 0.855 kGy h^{-1}. The cultures were re-cultured in liquid BG11 for evaluation of growth by measuring protein content /L culture according to method of Lowry et al. [23], and finally total carbohydrates according to Dubois et al. [24], amino acids contents according to Moore et al. [25, 26], in addition to superoxide dismutase activity which was estimated according to Bridges et al. [27].

3. Results and Discussion

Data (Figure 1) revealed that gamma irradiation recorded non-significant, differences in the total carbohydrate content of the two cyanobacterial species. Total carbohydrate content of *S. mundulus* recorded 490.21 and 519.83 mg g^{-1} at 1.0 and 2.0 kGy, respectively. *A. platensis* also recorded 298.667 mg g^{-1} at 1.0 kGy meanwhile 2.0 kGy dose and control samples recorded 274.451 mg g^{-1} and 243.429 mg g^{-1}, respectively

Data illustrated in Error! Reference source not found.) showed decrease in the protein content of *A. platensis* after exposure to gamma irradiation. Cultures of *A. platensis* displayed a significant reduction in protein content at both doses. Gamma irradiation treatment led to a significant decrease in the protein content of *A.*

platensis from 538.137 mg g^{-1} for control sample to 405.438 mg g^{-1} at 2.0 kGy irradiation dose, whereas, protein content in S. mundulus displayed non-significant difference recording 358.129 mg g^{-1} for the (control sample) , and 259.457 mg g^{-1} for the (2.0 kGy) treatment.

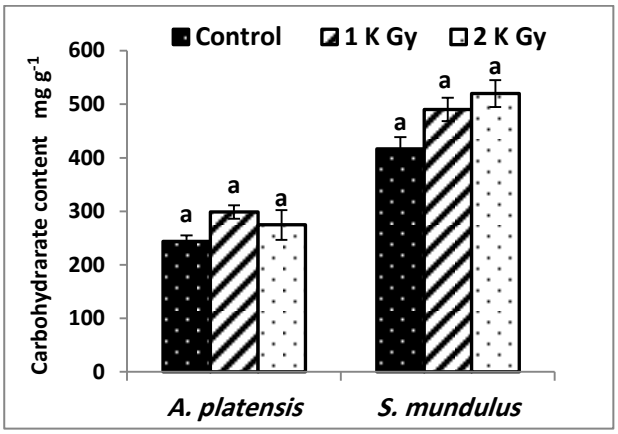

Figure 1 Total carbohydrate content of *A. platensis*, and *S. mundulus* cultures at stationary phase after treatment with γ- irradiation compared with the control culture. Data represent the mean of three replicates, and error bars represent the SD of these replicates. Columns with the similar letter exhibited insignificant difference (at $p \leq 0.05$).

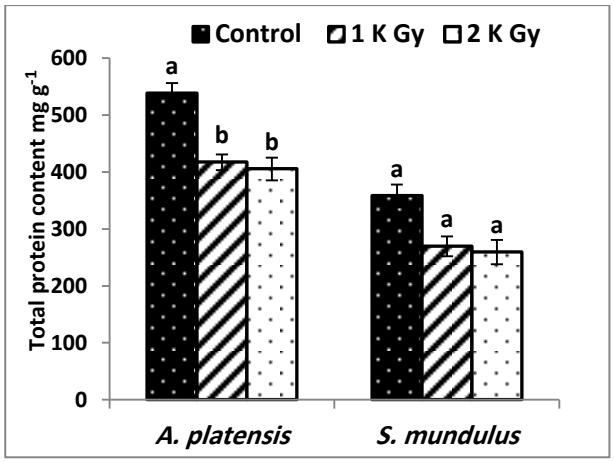

Figure 2 Soluble protein content of *A. platensis*, and *S. mundulus* at stationary phase after irradiation treatment for both γ-irradiated samples compared with control. Data represent the mean of three replicates, and error bars represent the SD of these replicates. Columns with the similar letter exhibited insignificant difference (at $p \leq 0.05$).

As shown in Figure 3), gamma irradiation at 2.0 kGy enhanced the total free amino acids in *A. platensis* whereas a significant increase was observed from 158.406 in control to 188.500 mg g^{-1} for 2.0 kGy, irradiation in *A. platensis*. While, *S. mundulus* did not record any significant difference in the total free amino acids content.

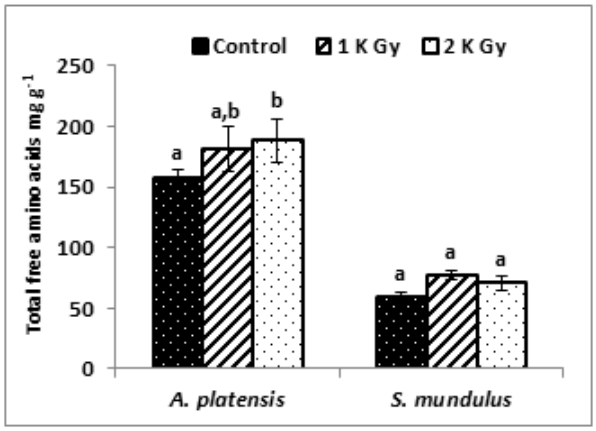

Figure 3 Total free amino acids content produced by *A. platensis*, and *S. mundulus* at stationary phase after irradiation treatment for both γ-irradiated samples compared with control ones. Data obtained from the mean of three replicates, and error bars represent the SD of these replicates. Columns with the similar letter exhibited insignificant difference (at $p \leq 0.05$).

The SOD activity of all irradiated *A. platensis* and *S. mundulus* cultures displayed a significant increment (**Figure 4**) compared to control cultures. Respecting *A. platensis* SOD activity was increased from 0.356 in non-irradiated cultures to 0.383 and 0.426 in response to 1.0 and 2.0 kGy irradiated cultures, respectively. Meanwhile 1.0 and 2.0 kGy *S. mundulus* irradiated cultures recoded SOD activity quantified as 0.287 and 0.243, respectively, while control *S. mundulus* cultures recorded 0.183. All these determination were performed compared to standard ascorbic acid which recorded 0.815.

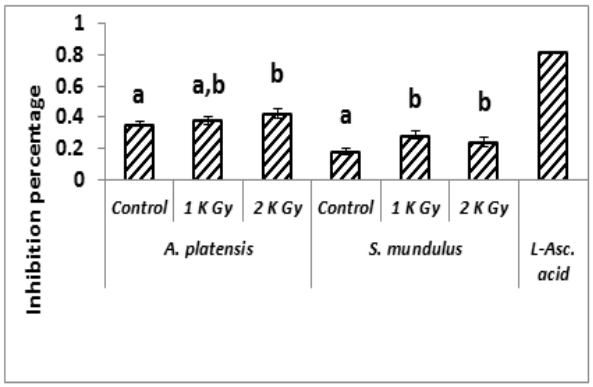

Figure 4 Superoxide dismutase activity for *A. platensis*, and *S. mundulus* at stationary phase

for both γ-stressed samples compared with control. Data represent the mean of three replicates, and error bars represent the SD of these replicates. Columns with the similar letter exhibited insignificant difference (at $p \leq 0.05$).

Discussion

There are two ways of interaction between γ-irradiation and biological system, direct and indirect. In the direct ways, resulted irradiation energy is dropped directly in the biological molecules meanwhile in the indirect one, energy is absorbed by the surrounding medium that cause the generation of diffusible radicals which attack the biological molecules [28]. As a result of the omnipresent water content of algal medium (about 99.9%), the major reactive oxygen species (ROS) quantity which generated in γ-irradiated cells had been resulted from water radiolysis, so the radiological effect of γ-irradiation was estimated by affecting the biomolecules and atoms in the cyanobacterial cells [21, 29]. Previous studies reported the effect of gamma irradiation on the total carbohydrate content of *Arthrospira* sp. [20] recorded a significant increment in carbohydrate content of irradiated *Arthrospira platensis* culture (about 248%) over the non-irradiated ones. A gradual increment in carbohydrate content of irradiated *spirulina platensis* till 2.0 kGy irradiation dose that increased by 84% in compared with non-irradiated cultures had been documented [30]. Also an increment in polysaccharides amount in the mutant *spirulina* cells with γ-irradiation meanwhile a slight decrease in protein content had been recorded using FTIR analysis [31]. Previous studies explained changes in protein content in both microbial and plant cells after exposed to different doses of irradiation, [32] reported that total soluble protein content of *Orthosiphon stamineus* plantlets had been significantly decreased in a dose dependent manner after 3 weeks from γ-irradiation with different doses (0-70) gray. [33] suggested that the inhibition in photosynthetic process occurred in irradiated cells of *Anacystis nidulans* during incubation period after irradiation could be a result of reduction of protein synthesis in thylakoid membranes, these decline in protein synthesis after γ-irradiation exposure may be as a result to direct toxic effect on translation or as a result to DNA damage. [34] reported that ionizing radiation enhanced an increment in some amino acids of green microalgae *(*Chlorophyceae) such as leucine, alanine and valine as result of cell protein lysis in reversible processes do not lead to cell death . [35] documented that SOD activity had been evaluated in *spirulina platensis* after irradiation with different gamma radiation doses ranged between 1.0 and 2.5 kGy. [36] interpreted this increment in SOD activity as a result of high altitude of toxic free radicals which act as a defense mechanism for radiation stresses.

Conclusion

This study indicated that gamma irradiation has a positive effect on total carbohydrate, total free amino acids content as well as SOD activity in both *A. platensis* and *S. mundulus* meanwhile protein content recorded a declining in twostudied organisms at both irradiation doses**5.**

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