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Gamma Irradiation: Impact on Chromate Resistant Cyanobacteria

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ABSTRACT: The effect of gamma rays on the growth parameters of two locally isolated chromium resistant unicellular cyanobacteria, having an affinity to the genus *Synechocystis sp.* [AHZ-HB-MK (DQ381960) and AHZ-HB-P2A (DQ398589)] were investigated. Both strains were irradiated at different stages of growth i.e. after 5, 15 and 30 days of culturing. Irradiation doses range from 0-20 Grays as generated by a gamma irradiator (Co^{60}). Chromium reduction potential was increased in AHZ-HB-MK (DQ381960) 5 and 15 days old culture after exposure to different doses of gamma rays, whereas no enhancement in reduction potential was observed in the case of AHZ-HB-P2A (DQ398589). There was a significant increase in chlorophyll content in 5 day old cultures of both strains at doses of 1 to 10 Grays. Carotenoid content increased significantly only in AHZ-HB-MK (DQ381960) sp. at all growth stages.

KEYWORDS: Gamma irradiation, *Synechocystis*, Cr (VI) reduction.

INTRODUCTION

Cyanobacteria (also called blue-green bacteria, blue-green algae, cyanophyceae, or cyanophytes) are a large and widespread group of photoautotrophic microorganisms, which originated, evolved, and diversified early in Earth's history. The earliest forms attributed to this group were found in sedimentary rocks formed 3.5 billion years ago, and it is commonly accepted that cyanobacteria played a crucial role in the Precambrian phase by contributing oxygen to the atmosphere[25].Algae, cyanobacteria and higher plants are unique in the biosphere since they can use light energy to split water molecules and evolve oxygen in a process that produce storable energy-rich products from atmospheric carbon dioxide and other inorganic nutrients. These activities are vital for maintaining the present levels of biomass on our planet and for sustaining an oxygenic atmosphere[17].

Environmental pollution by chromium may be severe. Chromium contamination is known to be prevalent at U.S. Department of Energy sites[23]. The electroplating and leather-tanning industries also contribute to environmental contamination with Cr (VI). Chromate compounds containing Cr (VI) are used widely in the cooling towers of heavy industry and atomic power plants, since Cr (VI) prevents corrosion and the growth of organisms [4]. Cr (VI) is soluble, toxic, and carcinogenic, whereas Cr (III) is less soluble and less toxic [12]. Thus, it is desirable to change Cr (VI) into Cr (III). This approach is taken in the bioremediation of Cr (VI) pollution. It shows promise for solving pollution problems and has advantages over various other physical and chemical methods. Chromate-reducing activities can be found in the cell extracts of many bacteria[5, 6, 7, 10, 13, 19]. Chromate reductase can reduce the toxicity of Cr (VI) by reducing it to Cr (III) and lowering its solubility[6, 9]. Microorganisms form a group of inseparable interacting communities, which are subjected to such unfavorable alteration of the aquatic and other ecosystems. Cyanobacteria, a group of prokaryotic, photosynthetic nitrogen fixers are present in every ecological niche and therefore, exposed to the toxic effects of the metals. The effects of a few metals have been studied with respect to growth, nitrogenase activity and carbon fixation,[14, 29, 3, 15, 16, 27, 1, 8, 28]. Some cyanobacterial species are known to reduce toxic Cr VI into less toxic Cr III[21]. In cyanobacteria, metal ion sequestration inside the cell is performed by the Class II metallothioneins. Class II metallothioneins are thiol-containing, cysteine-rich, metal-binding proteins that sequester metal, thus preventing accumulation of potentially toxic-free metal ions within the cell. Metal ion binding occurs through the interactions of the ions with the thiol groups of cysteine residues. The metallothionein genes are arranged as an operon called the smt locus, containing both smtA (metallothionein protein) and smtB (repressor, regulatory protein) genes. SmtB is a transacting repressor of expression from the smtA operator/promoter region. Metallothionein expression, from the gene to the functional protein, is induced by these metal ions and the regulation of transcription to messenger RNA is dependent upon the interaction

between these metal ions and the repressor protein regulating transcription, again via interaction with thiol groups present on the repressor protein. Loss of the smtB repressor gene and subsequent unregulated transcription of smtA may be advantageous to organisms constantly stressed with high levels of cadmium, copper, arsenic, and lead. Since cells devoid of functional repressor (smtB) show elevated concentrations of smtA messenger RNA transcripts even in the absence of an inducer.

The major objective of this study was the formation of mutants of Cr resistant cyanobacteria with increased reduction potentials by using irradiation mutagenesis. Ionizing radiations including gamma rays damage the photosynthetic apparatus and reduces the oxygen evolution efficiency of cyanobacteria[8]. They have the shortest length and the highest energy of any other radiation in the electromagnetic spectrum. At very high level gamma rays can denature proteins. At lower doses, gamma rays collide with various molecules (often water), producing highly reactive species, such as hydroxyl and hydride radicals. Gamma rays can also directly damage DNA, causing double stranded breaks and other mutation[17].

Materials and Methods

Strains and Culture Conditions

Two locally isolated chromate resistant cyanobacterial strains of *Synechocystis sp.* AHZ-HB-MK (DQ381960) and AHZ-HB-P2A (DQ398589)[11] used in this study are able to tolerate up to 200 µ g K_2CrO_4 ml^{-1} in BG 11 medium[23].

Irradiation Experiments

Strains were irradiated with gamma rays generated by a Co^{60} source from NIAB Faisalabad Pakistan. The dose ranged from 0.5 to 20 Grays. Treated strains were plated on BG 11 solid medium. The number of colonies was counted after 10 days. Five colonies were selected from each plate and grown in BG 11 liquid medium in standard growth conditions[11].

Growth Measurements

Growth of both strains was measured using chlorophyll a content as described later [24].

Pigment Analysis

To evaluate the effects of gamma rays on the photosynthetic pigments, extract were prepared in 100 % acetone and the amount of carotenoid (µg ml^{-1}) was calculated[18].

Chromium Reduction Experiment

To check the chromium (VI) reduction potential in the treated strains, BG 11 medium with an initial K_2CrO_4 concentration of 100 µg ml^{-1} was used. Cultures were harvested after 5, 15 and 30 days and the Cr (VI) reduction potential was determined[7]. The only modification in this method was the use of BG II medium instead of reduction medium.

Statistical Analysis

Standard error of the means and LSD (least significant difference) was calculated[26].

Results

Growth Measurements

Growth of both strains was measured in terms of chlorophyll a concentration (µg ml^{-1}) after exposure to different doses of gamma rays. Gamma rays caused a significant effect on chlorophyll a concentration of the treated strains when compared with the control (Fig 1). In the case of 5 days old culture, an increase in chlorophyll content at all doses was observed in both strains, where as in 15 and 30 days old cultures, a gradual reduction in chlorophyll content was observed.

Pigment Analysis

Carotenoid concentration was enhanced significantly in AHZ-HB-MK (DQ381960) at 1 to 10 Grays at all growth stages and in AHZ-HB-P2A (DQ398589) an increase was observed at 0.5 Gy in 5 and 15 days old culture and at 5 and 10Gy in 30 day old culture when compared with the control (Fig 2).

Chromium Reduction Experiments

At an initial concentration of 100 µg ml^{-1} of K_2CrO_4, AHZ-HB-MK (DQ381960) 5 days old culture showed to enhance reduction potential, 44.96%, 55.06%,47.36% and 49.06 % at 0.5, 1, 2, and 5 Grays respectively when compared with control (43.80 %). Similarly reduction potential was enhanced at 1 (85.20%), 2 (83.07%), and 5 (85.94%) Grays in 15 days old cultures when compared with control (77.8), while in 30 days old culture the reduction potential was decreased at all doses. AHZ-HB-P2A (DQ398589) showed increased reduction potential only in 5 days old culture at 0.5 (51.9%) and 1 (47.4%) Grays when compared with the control (42 %) (Table 1).

Discussion

Heavy metals are ubiquitous in the biosphere where they occur as part of the natural constituents of chemicals to which biota and human beings are frequently exposed. This results in introduction of substantial amounts of potentially toxic metals into the food chain. Microorganisms form a group of inseparable

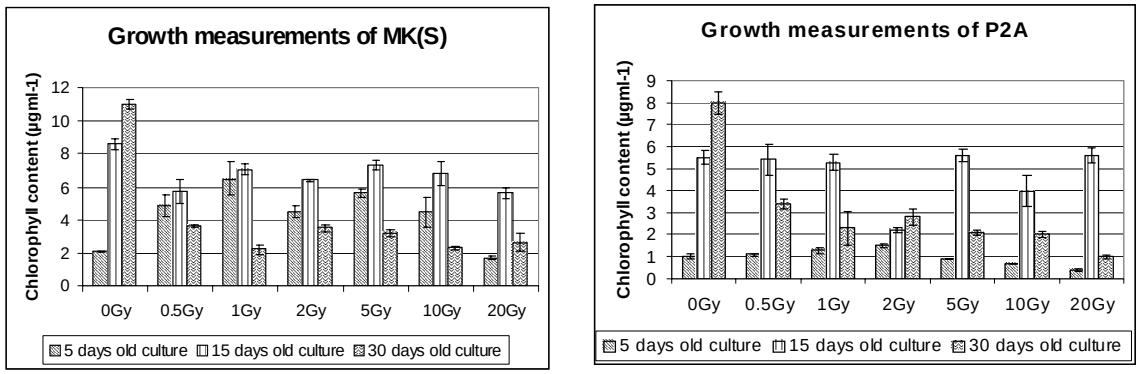

Fig 1. Growth measurements after exposure to different doses of gamma-rays, a. *Synechocystis sp.* AHZ-HB-MK (DQ381960), b. *Synechocystis sp.* AHZ-HB-P2A (DQ398589) [Bars represent standard error of the means (Mean of three replicates)].

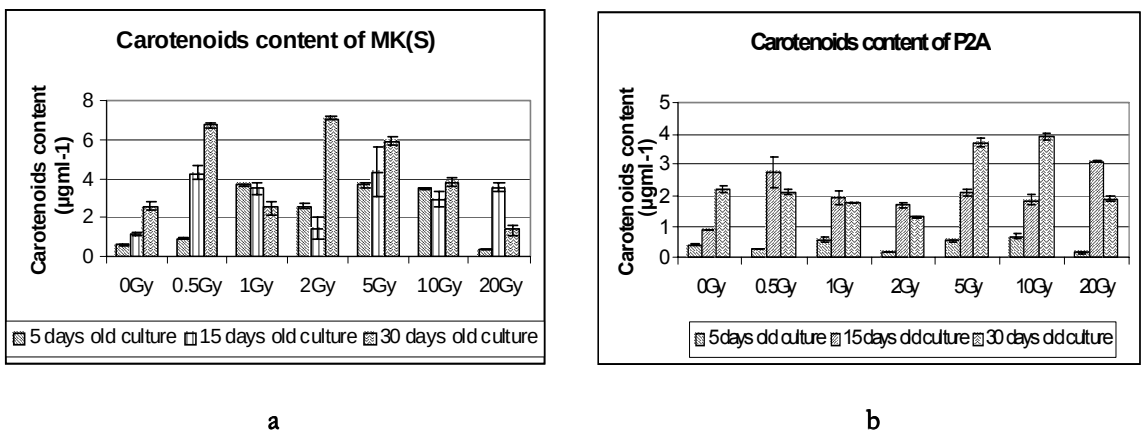

Fig 2. Carotenoid content after exposure to different doses of gamma-rays, a. *Synechocystis sp.* AHZ-HB-MK (DQ381960), b. *Synechocystis sp.* AHZ-HB-P2A (DQ398589).

Table 1. Cr (VI) reduction potential after exposure to different doses of gamma-rays.

Dose (Grays)	Cr (VI) reduction potential					
	5 days old culture		15 days old culture		30 days old culture	
	MK	P2A	MK	P2A	MK	P2A
0	43.80±1.50	42.00±0.98	77.80±0.68	79.41±0.81	92.70±1.01	87.54±1.79
0.5	44.96±1.43	51.90±0.23	76.83±0.30	79.49±2.61	63.36±0.43	64.96±1.69
1	55.06±3.27	47.40±1.42	95.20±1.44	70.62±5.27	76.96±1.71	63.21±0.79
2	47.36±3.32	40.50±1.19	93.07±3.03	71.86±0.57	72.99±1.68	64.44±0.58
5	49.06±3.36	41.20±0.20	95.94±0.60	76.18±1.63	68.38±2.20	57.43±0.93
10	22.53±3.19	42.60±0.18	96.04±1.23	78.12±0.82	67.14±1.03	55.89±0.39
20	24.10±1.38	44.41±1.27	93.86±1.57	73.36±2.47	57.62±2.63	55.64±0.75
LSD	(For treatments)11.69 (For strains)21.89		(For treatments)10.53 (For strains)19.69		(For treatments)5.13 (For strains)9.59	

interacting communities, which are subjected to such unfavorable alteration of the aquatic and other ecosystems. Cyanobacteria, a group of prokaryotic, photosynthetic nitrogen fixers are present in every ecological niche and therefore, exposed to the toxic effects of the metals. The effects of a few metals have been studied with respect to growth, nitrogenase activity and carbon fixation[17, 20, 27, 1, 8, 28]. The mechanism of toxicity of metals to cyanobacteria are not fully known but several heavy metals retard the flow of electrons in electron transfer reaction in mitochondria and chloroplast and thus can be expected to have a detrimental effect on respiration, photosynthesis and other processes related to it. This research paper points to the positive role of irradiation mutagenesis in reducing toxicity of chromium.

The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals, which can damage different important compounds of plant cell[20].

The present work focused on the determination of the effects of gamma rays on two locally isolated chromium resistant cyanobacterial strains, *Synechocystis* sp. AHZ-HB-MK (DQ381960) and AHZ-HB-P2A (DQ398589). AHZ-HB-MK (DQ381960) strain showed more resistance towards irradiation exposure as compared to AHZ-HB-P2A (DQ398589). It has also been observed that resistance toward ionizing radiation depends on the age of cells. At early stages of growth, the resistance mechanism was strong, whereas, at late stages of growth there is a gradual reduction in this mechanism.

Light energy is absorbed by pigments and the energy excites electrons to a higher energy level. The pigments are associated with proteins in the core complexes of the two photosystems. There are three classes of photosynthetic pigments in photosynthetic organism. UV-B and gamma irradiation damage these systems. To evaluate the effect of gamma rays on photosynthesis of both strains, chlorophyll a concentration was estimated. In *Synechocystis* sp. AHZ-HB-MK (DQ381960), 15 and 30 days old cultures, the amount of chlorophyll a decreased at all irradiation doses. In 5 days old culture, the concentration of chlorophyll a increased at all doses except 20 Gy. In AHZ-HB-P2A (DQ398589) 30 days old culture, chlorophyll a content also decreased at all irradiation doses, while in 5 and 15 days old cultures, there was no change in the concentration of chlorophyll a at any dose except 2 Gy, where chlorophyll a decreased significantly in 15 days old culture.

Carotenoids play a major role in the photo protection of cells and tissues. This ability is the result of energy transfer reactions in which the energy of triplet state sensitizers or singlet oxygen is transferred to carotenoid molecules in the ground state, forming triplet state carotenoid molecules. The energy acquired by the carotenoids is then lost as heat and the ground state carotenoid is regenerated to undergo another cycle of photo protection. It has been observed that twelve hours after the induction of irradiation stress, lipid bodies containing secondary carotenoids appeared around the chloroplast and accumulated at the periphery of the cell. After 3 days under light stress, the chloroplast was modified to a chromoplast-like organelle, full of secondary carotenoids and free of thylakoid membranes. The profile of the secondary carotenoids in the lipid bodies was similar to that in the chromoplast. After 3 days under stress, a hydrophobic layer rich in secondary carotenoids formed inside the cell wall. The lipid layer may function as a light filter to reduce irradiation of the cell components, to prevent photooxidative damage and to reduce water losses. The halotolerant alga *Dunaliella bardawil* accumulates very large amounts of beta-carotene when exposed to high light intensity. The accumulated beta-carotene is concentrated in small, oily globules within the chloroplast and has been suggested to protect the alga against photo damage by high irradiation. In this study, the amount of carotenoids was also increased at 2, 5, 10 and 20 Grays in both strains.

Cyanobacteria are highly adaptable organisms. These organisms can respond to changing environmental conditions such as temperature, light, and metal ion exposure. All organisms must possess mechanisms that regulate metal ion accumulation and therefore, avoid heavy metal toxicity while still procuring metals in trace amounts that are essential for normal cell growth. An important protective mechanism used by cells in response to a variety of stressors is the expression of heat shock genes. These proteins are present in highly conserved forms in all organisms studied including bacteria, plants, and animals. One of the most important of these heat shock proteins is GroEL. GroEL is a 58,000 amu protein which assembles into two stacked rings of seven subunits each with an additional ring of seven, 10,000 amu GroES subunits. Together, this complex has been shown to renature and make functional proteins that have been misfolded as a result of cellular stress. Cyanobacteria increase transcription of groEL in response to alterations in the environment to prevent protein aggregation and misfolding. In addition, these cells respond to a variety of metals ions and the oxyanions of arsenic by producing the metal-binding proteins called metallothioneins. It appears that this sequestration of these metals detoxifies them, thus decreasing their detrimental effects on the cell. Through genetic engineering, it will be possible to create cyanobacterial strains which express these proteins at very high levels, making such strains potentially valuable

for attempts at bioremediation. Chromium (VI) reduction potential has also been increased after exposure to irradiation. In AHZ-HB-MK (DQ381960) increase in reduction potential was observed at 0.5, 1, 2, and 5 grays in 5 days old culture, while in 15 days old culture, increase was observed at all doses. In AHZ-HB-P2A (DQ398589), increase has been observed only at 0.5 and 1 grays in 5 days old culture.

Hence from the above discussion it is concluded that some irradiation doses resulted in the production of certain mutants of cyanobacterial strains which showed improved ability in reducing the toxicity of hexavalent Cr into less toxic trivalent chromium. These mutants can be utilized in the bioremediation of Cr (VI) contaminated wastewater and soils.

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