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Survival of the Toxic Cyanobacterium *Microcystis panniformis* Komárek *et al.* Following Treatments with Gamma Radiation and Heating

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Abstract: It is proposed an original procedure for the population control of toxic cyanobacteria by associating gamma radiation with heating. *Microcystis panniformis* Komárek *et al.* was irradiated with 5 KGy of gamma radiation, immediately followed by a heating treatment at 40°C during 90 min. The results revealed that the combination of irradiation with the heating treatment increased the number of deaths relatively to irradiation alone. Actually, after 48 h from the beginning of the experiment the death percentages relatively to the control, in samples irradiated/heated and only irradiated, were 98 and 72%, respectively. It is our understanding that the use of gamma radiation associated with heating is a new technology, which could be adapted for large scale control of toxic cyanobacteria present in water supply reservoirs.

Key words: Aquatic environment, cyanobacteria, *Microcystis*, toxin, water treatment

Microcystis panniformis Komárek *et al.* (Cyanobacteria, Chroococcales) is a common tropical cyanobacterium responsible for toxic bloomings in euthrophic water bodies supplying human populations, leading to severe public health problems (Bittencourt-Oliveira *et al.*, 2005).

When penetrating a cell, gamma radiations generate free radicals and other reactive chemical species in the citosol, causing damages to several molecular targets. In particular, ionizing radiations are able to promote double strand break in DNA molecules which, when not successfully repaired, could lead to mutations or induction of cell death.

The crucial point associated with the population control of cyanobacteria is to investigate at which doses cell viability could be severely hampered while preserving membrane integrity and avoiding to release toxins to the aquatic environment. However, maintaining population sizes small may require the use of high doses of radiation, which could represent both a drawback and a shortcoming for its large scale use. The main motivation of this study is the search for a synergistic association of radiation with another non-radioactive physical agent, in order to achieve population control with much smaller doses. In this sense, we propose the use of radiation with heating treatment. Thus, while radiation produces damages in several intracellular targets, it is our expectation that a subsequent heating treatment would make cellular recuperation much more difficult.

The non-axenic *M. panniformis* BCCUSP100 strain was maintained under controlled culture conditions at 22±1°C, 30±2 μmol m^{-2} sec^{-1}, 14:10 h (light-dark) photoperiod in BG-11 medium (Rippka *et al.*, 1979) before and after the beginning of the treatments.

In the exponential growth-phase, fifty milliliters of culture sample with $8.5\pm0.5\times10^6$ cells mL^{-1} were exposed in triplicate to the following treatments, (a) treatment 1: gamma irradiation with a dose of 5 KGy at a rate of 0.89 KGy h^{-1}, from a ^{60}Co source (Gamma-cell 220, MDS Nordion, Otawa, Canada), followed by heating at 40°C during 90 min, (b) treatment 2: only gamma irradiation

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with a dose of 5 KGy at 0.89 KGy h^{-1} and (c) treatment 3: heating at 40°C during 90 min and d) Treatment C: control sample with no treatment.

The choice of the dose (5 KGy) was made after examining the survival dose-response curve of *M. panniformis* (data not shown). Cell densities were estimated after 24 h (T_1) and 48 h (T_2) from the samples exposure to the physical agents by counting the number of survivals. It was established that a minimum of 400 counted cells is required to obtain an error of approximately 10% at a confidence level of 95% (Guillard, 1973).

Table 1 show our final results, plus their mean values and standard devastations calculated by the conventional manner. The number of survivals in samples submitted only to heating treatment was 73% of the survivals in samples not submitted to any treatment (control) at T_1. A discrete recovery was observed at T_2.

The effect of the irradiation treatment was slightly more cytotoxic than heating at T_1. Interestingly however, at T_2 the percentage of survivals (28%) is about half the one at T_1 (68%). Submitting the samples to irradiation, followed immediately by heating, increased cytotoxicity appreciably at T_1 (44%), but it was almost 100% lethal after 48 h (T_2), where only 2% of survivals were observed.

From the results, the role played by the time elapsed since completion of the treatments was not noticed in the heating treatment (Treatment 3), but it was important in the treatment with gamma irradiation (Treatment 2), since from T_1 to T_2 cell viability decreased from 68 to 28%. This is consistent with a scenario of moderate thermotolerance for the *M. panniformis*.

On the other hand, in the irradiation plus heating treatment (Treatment 1) the time effect was overwhelming, since cell viability decreased from 44% to 2% from T_1 to T_2. Such delayed effect could be probably due to the fact that the radiation damages inflicted by gamma radiation are mostly indirect, that is, they are originated from free radicals produced by the radiolysis of water (Chaterjee, 1989). These free radicals reach the targets through slow and passive diffusion in the citosol.

It comes evident that after 48 h (T_2) the irradiation plus heating treatment is at least one order of magnitude more cytotoxic than the irradiation alone. Considering that the heating treatment alone is only responsible for a modest cytotoxic effect, we come to the conclusion that the role of heating in the irradiated samples was predominantly the one of an interfering physical agent in the cell recuperation processes. These findings suggest that, since DNA is the bigger and more important cellular target, the heating treatment also negatively interfere with DNA repair mechanisms.

Table 1: Number of surviving cells ($\times 10^6$) and the calculated standard deviations for *M. panniformis* after 24 and 48 h from the samples exposure to the treatments

Treatments	24 h (T_1)	48 h (T_2)
Irradiation plus heating	4.3±0.660	0.2±0.09
Only irradiation	6.6±1.260	2.8±2.78
Heating	7.1±0.374	8.0±0.97
Control	9.7±0.210	10.0±0.07

M. panniformis is quite resistant to gamma radiation in comparison with other prokaryotes (Taghipour, 2004; Thompson and Blatchley, 2000). So its population control requires high doses of ionizing radiation, with the undesirable effect of membrane rupture. Only with the use of much lower radiation doses membrane integrity could be preserved, which could be achievable by the combination of irradiation with heating treatment, an evident advantage that could lead to the development of new techniques and strategies for large-scale treatment of water from natural reservoirs.

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