Evaluation of *Escherichia Coli* Cells Damages Induced by Ultraviolet and Proton Beam Radiation

Jaqueline Kappke[1], Edilsa Rosa da Silva[1], Hugo Reuters Schelin[1], Sergei A. Paschuk[1], Artem Pashchuk[1], Analisa de Oliveira[1], Nelson Carlin Filho[2], Eloisa Madeira Szanto[2], Jun Takahashi[2], and Jairo Cavalcante de Souza[2]
[1] *Federal Center of Technological Education - CEFET/PR, Av. Sete de Setembro, 3165, CEP 80230-901, Curitiba, PR, Brazil and*
[2] *PELLETRON Laboratory, Physics Institute of São Paulo University, Brazil*

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Prokaryote cells were exposed to ultra violet (UVc) radiation and to proton beams in order for the induced effects to be studied. Morphological and physiological alterations occurred in *Escherichia coli* (*E. coli*) cells exposed to the beams were investigated. The measurements using UVc radiation were made at the Biology Department of CEFET-PR while the measurements using proton beams were made at the Pelletron Accelerator of the Physics of the University at São Paulo. An exposition time of 3 to 15 seconds for UVc radiation and dose ranging from 0.2 to 10.0Gy for protons was used. A cellular survival curve versus exposition time and absorbed dose was built for each case. After the irradiation the cells were submitted to a series of biochemical tests. It was observed that the *E.Coli* cells lost some basic biochemical properties when the received doses were in the range of 0.2 to 0.7Gy. By microscopic observations it was noticed that the *E.Coli* cells elongated after irradiation with UVc as well as with proton beam.

I. INTRODUCTION

The prokaryotes compose an interesting group of microorganisms, which can be used as instruments of scientific investigation. This can be explained by the fact that they possess intrinsic properties, such as reduced time of generation and relatively low cost of culture and maintenance [1], [2].

The *Escherichia coli* (*E. coli*) is a common bacterium of the intestinal tract of warm-blooded animals. It is an important biotechnological tool, which makes it possible to obtain important parameters for the metabolic and genetic characterization of cells of more complex organisms. For example, *E. coli* can be used for the study of the radiation effects, which are caused in tumoral cells by therapeutical treatment [1], [2], [3].

The ionizing and not ionizing radiations can cause mutations through direct or indirect action on the cellular surface. Some mutations are undesirable and even lethal, however, some can be interesting for the survival of a species [1], [2], [4].

At the present work possible transformations on *E. coli* are evaluated. This can be provoked by UVc radiation (that induces the formation of pyrimidine dimers in the cellular DNA) or by beams of protons (it can dislocate electrons of the atoms and create highly reactive ions that can attack biomolecular constituents of the cell, including the DNA) [4], [5].

These experiments were made with UVc radiation, at the Biology Department of Federal Center of Technological Education of Parana (Curitiba, Brazil), and with beams of protons, at the Pelletron Accelerator of the Physics Department of the University of São Paulo (São Paulo, Brazil).

The occurrence of morpho-physiologic alterations in the cells of *E. coli*, after-effects of irradiation and determination of the tax of cellular survival were also investigated.

II. METHODOLOGY

Cells of *E. coli* were cultivated in nutrient broth (pH 7.0, 36^oC) for 24 hours. The cells were then centrifugated (3500rpm, 15 minutes) and resuspended in solution of NaCl 0.85%. This solution was used to make the dilutions of which 0.05mL were inoculated in Agar MacConkey, selective for gram-negative bacteria. For the irradiation with UVc radiation a dilution of 1 : 10000mL was used, and for the proton beam, a dilution of 1 : 1000mL was used. The Petri plates (triplicate) which contain the microorganisms in Agar MacConkey were irradiated [3], [5]For ultra violet radiation, an exposition time of 3 to 15 seconds was used in a laminar flow chamber. For the radiation with proton beams the absorbed doses ranged from 0.2 to 10.0Gy.

The radiated Petri plates containing the microorganisms were cultivated during 24 hours (36^{o}C) the number of colonies formed were later determined. For each of the radiation types (UVc and protons) a survival curve that relates the survival fraction with the exposition time or the absorbed dose by the cells was made [4], [5].

The radiated cells were then submitted to biochemical tests and also to microscopic analysis (Gram-stain) in optical microscope (magnification of 1000x). The biochemical test carried out was the following: Vogues-Proskauer, Indol production, descaboxilation of L-lisina, glucose fermentation, lactose fermentation, rhamnose fermentation, urea hydrolysis, H_2S production, citrate and ornitine [3], [5].

III. RESULTS AND DISCUSSION

After a 24 hour (36^{o}C) incubation period, the Petri plates (MacConkey agar) containing irradiated microorganisms were evaluated and the colony formed units (CFU) in each plate were counted. The average number of CFU for each ex-

position time was calculated through equation 1 concerning survival fraction (FS), where N represents the CFU after each irradiation and N_0 corresponds to the CFU in plates not exposed to radiation [6], [7]:

$$FS = \frac{N}{N_0}; \quad (1)$$

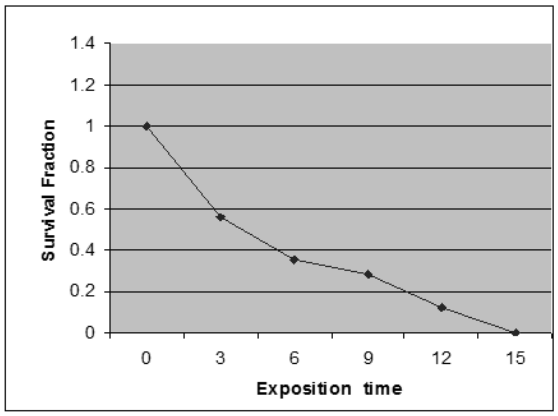

FIG. 1: Survival curve of the *E. coli* submitted to UVc radiation.

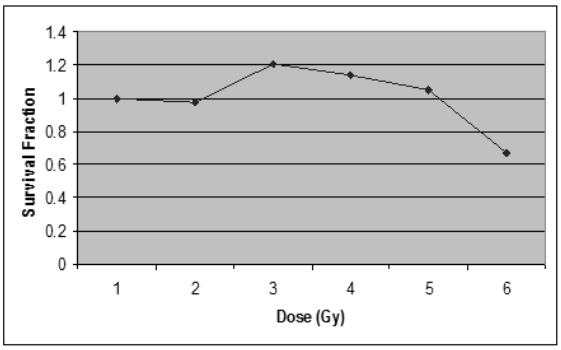

FIG. 2: Survival curve of the *E. coli* submitted to proton radiation.

Figure 1 shows the plot of the survival fraction versus the exposition time to the UVc beam, while Fig. 2 shows the plot of the survival fraction versus the absorbed dose of the proton beams.

In the Gram-stain the *E. coli* is visible as a short and red rod [2], [3], [8] as it is shown in Fig. 3 below (*E. coli* not radiated). The microscopical studies showed that the *E. coli* cells elongated after irradiation. The *E. coli* cells irradiated with UVc showed to be very elongated as can be seen in Fig. 4. As for the exposition with proton beam, it was also possible to observe that the cells were elongated as can be seen in Figs. 5 and 6.

In the literature the elongation phenomenon of *E. coli* cells was observed when it received a chemical treatment with cefalexin, cefalosphorin antibiotic of the betalactamic group. This antibiotic intervenes in the synthesis of the peptidoglycan of

FIG. 3: Gram-stain micrography of not irradiated *E. coli* (magnification of 1000x).

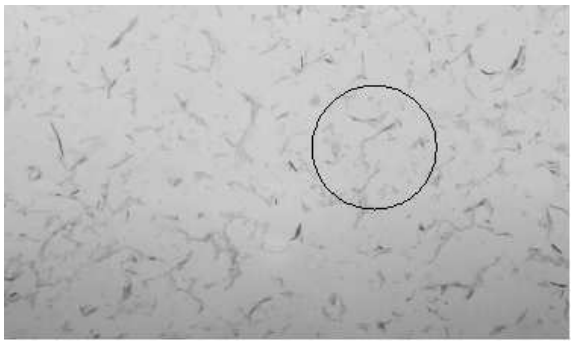

FIG. 4: Gram-stain micrography of *E. coli* exposed to 6 seconds of UVc radiation (magnification of 1000x).

the cell's wall, resulting in the incapacity of the cell to divide, without provoking its death [8].

The existence of the so called hormesis phenomenon is also registered, with stimulated or benefic effect, induced by low doses of a radioactive agent. The concept of hormesis radiation is normally applied to radiation doses in the range of 1 to 50*Gy*, which means, radiation doses that instead of provoking irreparable damage that would lead to cellular death, would provoke a radioadaptative response that would benefit the cell and its descendant [9], [10].

In the present work it was verified that the *E. coli* cells changed some characteristic biochemical properties when they received absorbed doses of 0.2, 0.4 and 0.7*Gy* as the loss of the capacity to descarboxilate the L-lisine aminoacid, as well as showing the cell elongated phenomenon related previously.

IV. CONCLUSIONS

With the experiments described above it was possible to conclude that:

* The 15-second exposition time with UVc radiation provoked a considerable reduction in the *E. coli* population;

* After the irradiation with UVc radiation, the *E. coli* cells did not present detectable metabolic alterations through the biochemical tests used in the present work;

* As for the proton beam irradiaton a decrease in the survi-

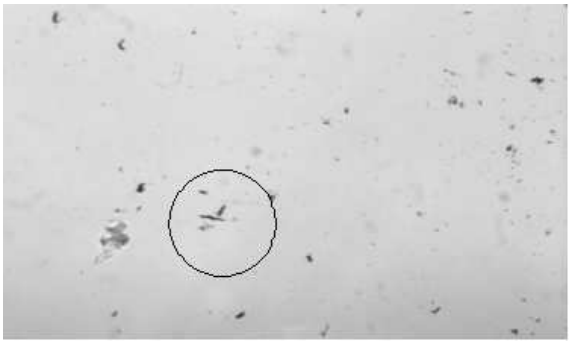

FIG. 5: Gram-stain micrography of *E. coli* irradiated with a dose of 0.4*Gy* with proton beams (magnification of 1000x).

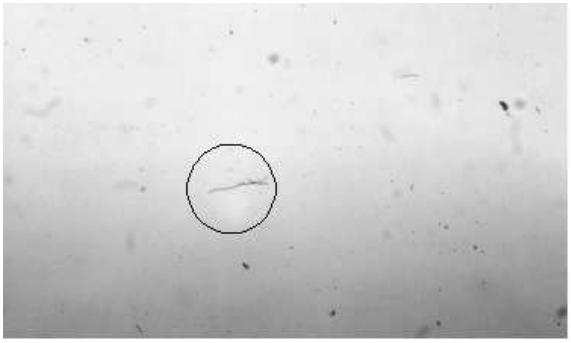

FIG. 6: Gram-stain micrography of *E. coli* irradiated with a dose of 0.7*Gy* with proton beams (magnification of 1000x).

val fraction for the applied doses of radiation was not observed. The tests should be rechecked and it would perhaps be necessary to increase the corpuscular radiation dose (proton beams) applied to the *E. coli*;

* The small radiation doses used (0.2, 0.4, 0.7*Gy*) for proton beams provoked alterations in the cellular metabolism of the *E. coli*, detected by the loss of capacity of the bacterium in descarboxilating the l-lisine;

* The *E. coli*, after exposed to a not lethal dose of radiation, UVc and protons, presented a change in its morphology, resulting in a well defined elongation.

Acknowledgments

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