STUDY OF *ESCHERICHIA COLI* CELLS EXPOSED TO PROTON AND GAMMA RADIATION

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We investigated alterations occurred in samples of Escherichia coli cells exposed to gamma rays and proton beams. The proton irradiation was made at the Pelletron Accelerator at USP and the gamma irradiation with a ^{60}Co Gammacell source at UFRJ. The delivered doses with protons were in the range of 0.2 to 6.0 Gy and with gamma radiation in the range of 30 to 780 Gy with a dose rate of 60 Gy/min. The analyses were made at UTFPR after the irradiation. A cellular survival curve as a function of the absorbed doses was obtained for both cases. The samples were also analyzed with Gram coloration, biochemical tests in EPM, MIO and Lysine broth, Simmons citrate medium and rhamnose broth. It was observed that for the received doses the E. coli cells presented morphological and biochemical alterations. The microscopic studies showed that the E. coli cells lenghtened after the irradiation with proton beams. The biochemical alterations found in this study were in lysine and EPM medium.

I. INTROTUCTION

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. The number of interactions and mutations is proportional to the absorbed dose, but the effects provoked by the interaction of the ionizing radiation with the biological tissues also depend on the kind of radiation and the exposure time. It may depend also on the state in which the cell is in its cellular cycle and the oxygen concentration in the radiation environment.[1]

The prokaryotes compose an interesting group of microorganisms, which can be used as instruments of scientific investigation. They possess intrinsic properties, such as reduced time of generation and relatively low cost of culture and maintenance. 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.

E. coli cells exposed to UV radiation in a previous experiment showed that an exposition time of 15 seconds was lethal for them and some morphological alterations, such as lengthening were verified.[2]

The present work intends to verify the occurrence of transformations in *E. coli* (ATCC 25922) exposed to gamma radiation (^{60}Co source at COPPE-UFRJ) and proton beam (Pelletron-USP). Morphological alterations in the irradiated cells will be checked through optical microscopy and analysis of the characteristics of the bacterial colonies. The action of the irradiated microorganism through biochemical tests will also be checked, verifying the alterations on the biochemical behaviors of the irradiated cells in comparison with the non-irradiated cells.

II. METHODOLOGY

II.A. Proton Beam

II.A.1. Sample Preparation

Cells of *E. coli* were cultivated in nutrient broth (pH 7.0, 36°C) for 24 hours. The cells were then centrifuged (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 the proton beam, a dilution of 1 : 1000mL was used.

II.A.2. Sample Irradiation

The Petri plates (triplicate) which contain the microorganisms in Agar Mac-Conkey were irradiated.[3] For the radiation with proton beams the absorbed doses ranged from 0.2 to 6.0Gy.[4-5] Fig. 1 shows the Petri plate irradiation with the proton beam.

II.A.3. Survival Curve

The irradiated Petri plates containing the microorganisms were cultivated during 24 hours (36◦C) and the number of colonies formed was later determined. A survival curve that relates the survival fraction (Eq. (1)) with the absorbed dose by the cells was made[4].

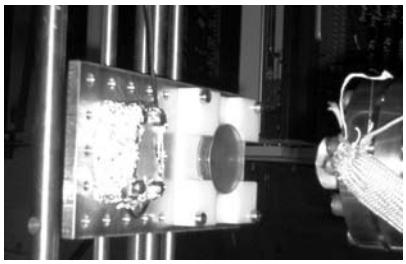

Fig. 1. Proton beam irradiation of a Petri plate at the Pelletron Accelerator -USP.

Eq. (1) determines the survival fraction (FS):

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

Where N is the number of colonies in irradiated Petri plates and N_0 is the number of colonies in the non-irradiated Petri plates.

II.A.4. Morphological Analysis

The irradiated cells were then submitted to biochemical tests and microscopic analysis (Gram-stain) in an optical microscope (magnification of 1000x).

II.A.5. Biochemical Analysis

The biochemical test carried out was the following: Vogues-Proskauer, Indol production, descaboxilation of L-lysine, glucose fermentation, lactose fermentation, rhamnose fermentation, urea hydrolysis, H_2S production, citrate and ornitina.

II.B. Gamma Irradiation

II.B.1. Sample Preparation

Cells of *E. coli* were cultivated in BHI broth (pH 7:0, 36°C) for 24 hours. The cells were then centrifuged (3500rpm, 15 minutes) and resuspended in solution of NaCl 0:85%. This solution was used to make the dilutions, of which 0.02mL of 1: 100000 were inoculated in Agar MacConkey, selective for gram-negative bacteria.

II.B.2. Sample Irradiation

After scattering the aliquots, the bacteria were submitted to gamma radiation (^{60}Co) with doses of 30, 60, 90, 120, 150, 180, 210, 240, 300, 480 and 780 Gy in triplicate. Non-irradiated plates were used as growing standards of *E. coli*. The irradiator used was the Gammacell 220 Excel (GC-220E) from the COPPE-UFRJ, as shown in Fig. 2, with a dose rate of ~60Gy/min.

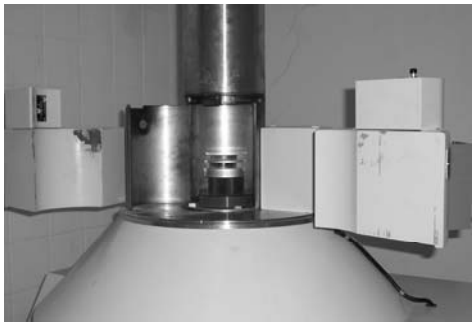

Fig. 2. The Gammacell irradiator (GC-220E).

II.B.3. Survival Curve

The irradiated Petri plates containing the microorganisms were cultivated during 24 hours (36◦C) and the number of colonies formed was later determined. A survival curve that relates the survival fraction (Eq. (1)) with the absorbed dose by the cells was made.

II.B.4. Morphological Analysis

The cultural characteristics of the microorganisms were evaluated concerning the shape (heterogeneous/ homogeneous), size, structure, texture and pigmentation of the colony and compared to non-irradiated microorganisms.

Microscopic examinations were made for classification and shape purposes (Gram staining).

II.B.5. Biochemical Analysis

The physiological characteristics of the irradiated microorganisms were evaluated through biochemical tests. The cells were initially cultivated in nutrient agar and incubated at 37°C for 24 hours.

Aliquots were inoculated in the specific environment medium for each of the biochemical test. The kit used in this analysis consisted of a set of five cultural medium to

identify the enterobacteria, which are: EPM Medium, Lysine Broth, MIO Medium, Simmons Citrate Medium and Rhamnose Broth[6].

III. RESULTS

III.A. Proton Irradiation

III.A.1. Survival Curve

Fig. 3 shows the plot of the survival fraction versus the absorbed dose of the proton beams (with 10% of error).

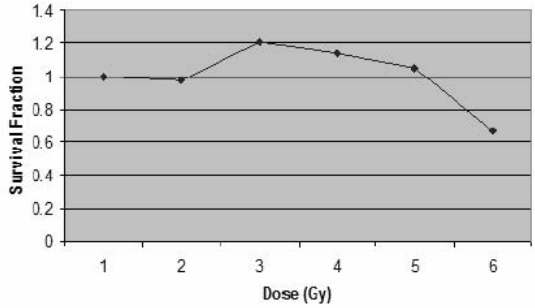

Fig. 3. Survival curve of proton beam irradiated cells.

III.A.2. Morphological Analysis

In the Gram-stain the *E. coli* is visible as a short and red rod (*E. coli* not radiated). The microscopic studies showed lengthening of the *E. coli* cells after irradiation as can be seen in Fig. 4 and 5.

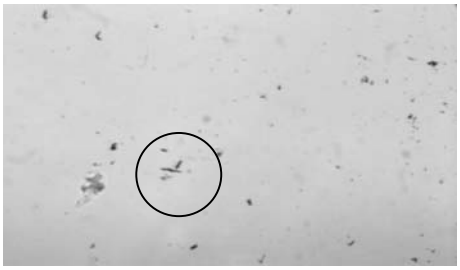

Fig. 4. Gram-stain microscopic examination of *E. coli* irradiated with a dose of 0.4*Gy* with proton beams (1000x magnification).

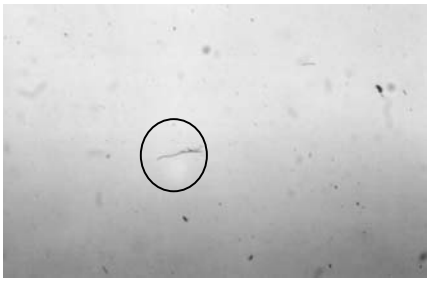

Fig. 5. Gram-stain microscopic examination of *E. coli* irradiated with a dose of 0.7*Gy* with proton beams (1000x magnification).

III.B.3. Biochemical Analysis

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 0.7Gy like the loss of the capacity to descarboxilate the L-lysine aminoacid, as well as showing the cell lengthening phenomenon related previously.

III.B. Gamma Irradiation

III.B.1. Survival Curve

Fig. 6 shows the plot of the survival fraction versus the absorbed dose of the gamma beams.

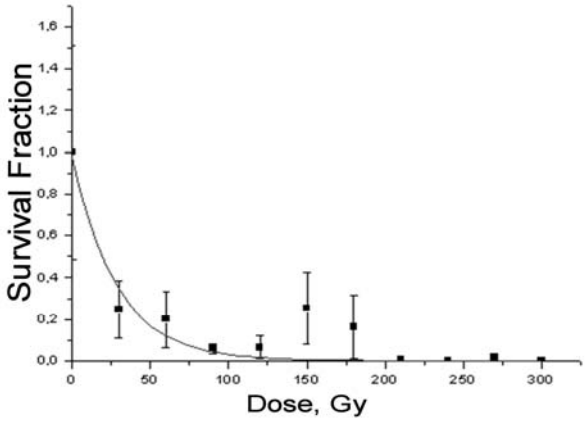

Fig. 6. Survival curve of gamma irradiated cells.

After the irradiation the *E. coli* showed to be able to survive with doses up to 210Gy being cultivated in MacConkey Agar at 37ºC. When exposed to radiation in food, the necessary dose for a 90% reduction of the *E. coli* cells is verified with doses around 260 Gy. Probably the culture medium (selective MacConkey Agar) utilized for the *E. coli* affected its sensitivity to the gamma radiation.[7]

III.B.2. Morphological Analysis

The characteristics of the *E. coli* irradiated and cultivated in positive lactose MacConkey Agar showed an intense pink-reddish coloration, dry and opaque with yeast smell, typical characteristics of the *E. coli* when cultivated in this medium.

There were no alterations observed in the microscopic characteristics of the *E. coli* cells after the irradiation with gamma ray beams. The *E. coli* before and after irradiation showed themselves as very short rods, as expected. The opposite of what was observed for the *E. coli* exposed to smaller doses with proton beams and with UV light.

III.B.3. Biochemical Analysis

When submitted to several biochemical tests the *E. coli* irradiated with different gamma radiation doses (^{60}Co) showed characteristic behavior of the non-irradiated *E. coli*. The only exception was the deamination of L-triptophan test, through the triptophan deaminase enzyme [12]. Fig. 7 shows *E. coli* non-irradiated in biochemical tests, and Fig. 8 shows *E. coli* irradiated with gamma rays.

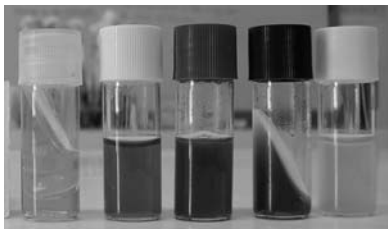

Fig. 7. EPM Medium, Lysine Broth, MIO Medium, Simmons Citrate Medium Rhamnose Broth, inoculated whith the non-irradiated *E. coli*.

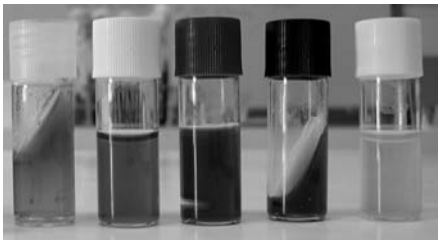

Fig. 8. EPM Medium, Lysine Broth, MIO Medium, Simmons Citrate Medium Rhamnose Broth, inoculated with the irradiated *E. coli*.

IV. CONCLUSIONS

As for the proton beam irradiation, a decrease in the survival fraction for the applied doses of radiation was not observed. The tests should be rechecked and it would perhaps be necessary to increase the radiation dose (proton beams) applied to the *E. coli*.

Through the performed analysis it was possible to show that a 240 Gy dose was lethal for the *E. coli* cells cultivated in MacConkey Agar irradiated by gamma rays.

The *E. coli*, after exposed to a non lethal dose of proton beam, presented a change in its morphology, resulting in a well defined elongation.

Microscopic morphological alterations were not detected in gamma irradiated *E. coli* cells, and macroscopic alterations in the colonies were not verified either.

The small radiation doses used (0.2, 0.4, 0.6*Gy*) for proton beams bring forth alterations in the cellular metabolism of the *E. coli*, detected by the loss of capacity of the bacterium in descarboxilating the l-lysine;

The performed biochemical tests showed that the gamma irradiated and the non-irradiated *E. coli* cells had the same behavior in the cultural medium Lysine Broth , MIO Medium, Simmons Citrate Medium and Rhamnose Broth. However, after the irradiation, the *E. coli* changed its behavior for the EPM Medium, becoming able to deaminate the L-triptophan.

It was possible to verify that the gamma radiation in non-lethal doses showed to be able to make alteration in the biochemical behavior of the *E. coli*.

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