Armenian Journal of Physics, 2016, vol. 9, issue 1, pp. 80-82

Computational analysis of ionizing radiation-induced shape parameter changes on bacterial cell

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Received 15 January 2016

Abstract. The purpose of this work was computational analysis of ionizing radiation-induced shape parameter changes on bacterial cell. For our investigation we have radiated Escherichia coli and Pseudomonas aeruginosa. Strains of Escherichia coli were exposed to UV light for 10 and 15 minutes, Pseudomonas aeruginosa were exposed to gamma radiation for 15 and 30 minutes respectively. The results of radiation showed that gamma radiation causes decrease of area and perimeter and increase of shape parameter. Results for Escherichia coli were a little different after 10 minutes area and perimeter were decreased compared with control, shape parameter was increased but after 15 minutes area and perimeter were increased, shape parameter was decreased.

Keywords: Bacterial cell, radiation, area, perimeter, shape parameter

Introduction

Radiation is one of the commonly employed method for the destruction of microbial cells. The prokaryots are interesting group of microorganisms. They possess intrinsic properties, low cost of culture and maintenance so can be used as a tool for the scientific investigations to obtain important parameters [1]. For example, using of bacterial cells as biosensor to sense the effect of ionizing radiation biologically, such as *Escherichia coli* (a common bacterium of the intestinal tract of human and animals)[2]. Microorganisms can be inactivated directly or indirectly by radiation due to impairment of cytoplasmic membrane [3].

Materials and Methods

Effect of ionizing radiation induced by UV lamp and γ-rays (Ra-226) on *Escherichia coli* and *Pseudomonas aeruginosa*was investigated. Radiation induces intracellular oxidative damage through the production of reactive oxygen species. Microorganisms were cultivated in nutrient broth and incubated at 37°C. Cells were irradiated with γ-rays and UV lamp, one ml of cell broth suspension was distributed in nutrient agar plate and incubated at 37°C.

Shape parameter changes induced after ionizing radiation were studied using computational analysis. For our study, we used software LabView permitting us to turn the source image obtained from polarized light microscope to input data for software “Nova”. Obtained optical images were preliminarily analyzed using Nova's Section mode by filtering after magnification or analyzed the distortions of pixels. Grain Analysis mode represents a source image, a section of the source image, a table of geometrical parameters of bacterial cell (area, average size, perimeter, length, volume etc.) and a histogram of distribution density of one of the parameters of grains. For our study, we use only area, average size and perimeter parameters. Importing derived geometrical parameters into MS Excel, we determine shape parameter offered by us. Shape parameter is equal to the ratio of the area to the square of the perimeter (Eq. 1).

$$\alpha = \frac{S}{P^2} \qquad (1)$$

Results and Discussion

LabView allows us also to study the degree of crystallization of bacterial cell membrane. It is known that when the polarizer and analyzer of the Polarized Light Microscope are crossed appears dark bank. If the sample contains crystal particles, the particles rotate the plane of polarization of the passing light and starting from a definite value or the rotation angle sufficient intensity of the light passes by the analyzer and is recorded by the receiver (video camera) and as much is the output intensity of the light as high is the degree of crystallization of the sample.

Samples were placed under radiation source (UV lamp) for 2, 5, 10, 15, 30 minutes and the dose rate was 40 Gy (Fig. 1).

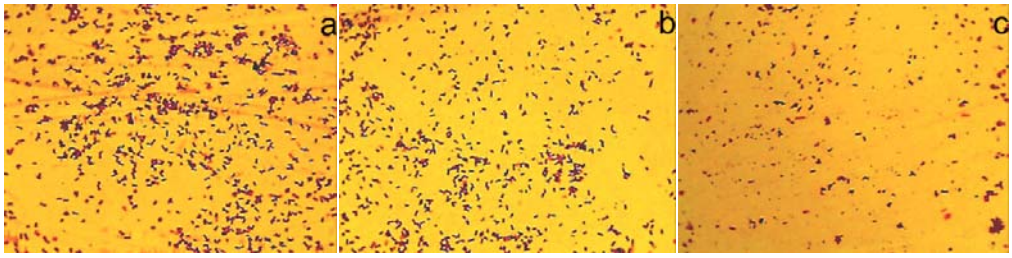

Fig. 1.UV radiation induced bacterial cells. Views of cells under microscope in control (a),10 minute radiated by UV lamp(b), 15 minute radiated by UV lamp(c).

As we radiated bacterial cells in their membranes occurred fluidity alterations, they become more permeable due to lipid peroxidation and protein denaturation and it leads to inflow of hydrophil substrates. The results indicate that the mean value of area in control was 19.57 nm^2 and the mean value of perimeter was 14.54 nm, as a result of 10 minute irradiation the mean values of area and perimeter were 14.3 nm^2 and 11.34 nm respectively, and the mean value of shape parameter increased from 0.093 to 0.11. In case of 15-minute irradiation the mean value of area and perimeter compared with control were in18.2 nm^2 and 13.8 nm, respectively, here the value of shape parameter was 0.096.

Using the same methods, we also radiated *Pseudomonas aeruginosa* 15 and 30 minutes using gamma radiation. Pictures derived from light microscope are shown in Fig. 2.

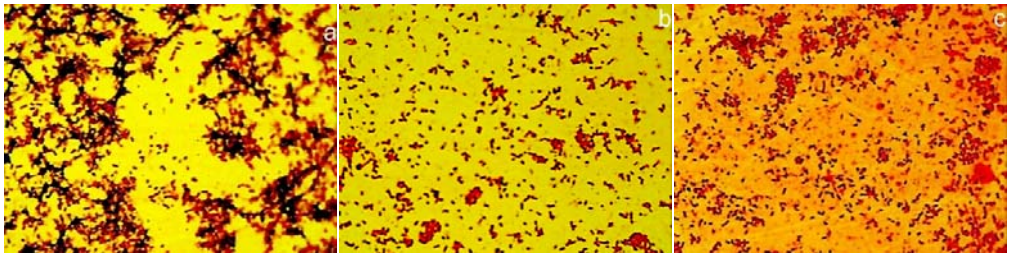

Fig.2.Gamma radiation induced bacterial cells. Views of cells under microscope in control (a),15 minute radiated Ra-226 (b), 30 minute radiated Ra-226 (c).

As a result of control we derived, data showing that the mean values of area and perimeter were 20.91 nm^2 and 14.8 nm, and the value of α was 0.098. After irradiation, values of area, perimeter and shape parameter were 18.15 nm^2, 13.66 nm and 0.098, respectively(values of 15 minute radiated) and 18.1 nm^2, 13.95 nm and 0.093(values of 30 minute radiated) (Figure 3,4). These results indicate that gamma radiation causes decrease of area and perimeter and increase of shape parameter.

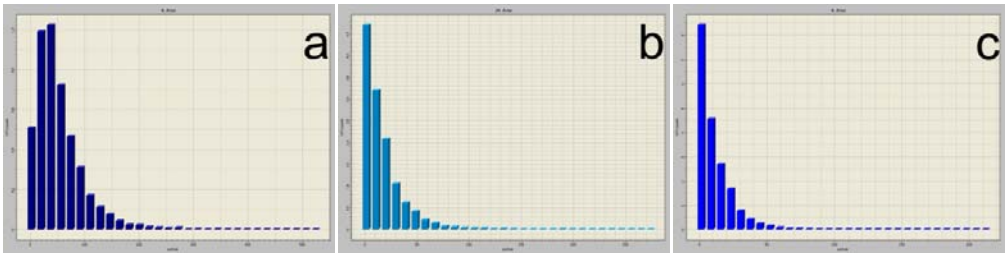

Fig.3.γ-radiation induced changes of bacterial cell areas in control (a),15*min* radiated (b), 30*min* radiated (c).

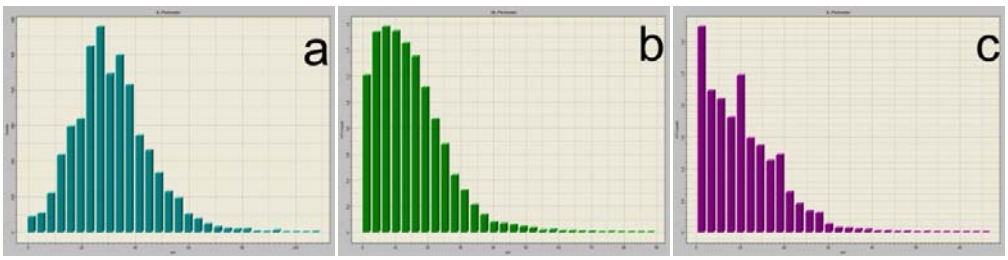

Fig.4.γ-radiation induced changes of bacterial cell perimeters in control (a), 15*min* radiated (b), 30*min* radiated (c).

This technique shows that data derived from computational analysis are more reliable. On the one hand it gives us opportunity to study hundreds and even thousands of bacterial cells, on the other hand analyzing is fast and effective.

Conclusion

In conclusion, we find that low dose irradiation (15 minute) for *Pseudomonas aeruginosa* causes decreasing of their areas by 13,2% and perimeters by 7,7%, longer irradiation (30 minute) causes 13,4% and 5,7%, respectively. For *Escherichia coli* we find that low dose irradiation (10 minute) causes decreasing of their areas by 26,9% and perimeters by 1,4%, longer irradiation (15 minute) causes 7% and 5%, respectively. A new shape parameter was suggested, which determines the dose dependent shape changes of cells.

Acknowledgements

This report was presented at ISTC International Workshop “Ionizing and Non-Ionizing Radiation Influence on Structure and Biophysical Properties of Living Cells” held in Tsakhkadzor, Armenia on September 25-27, 2015 with support of ISTC in the framework of research project A-2089.

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