AFFECT OF ULTRASHORT ELECTRON BEAMS ON THE *ESCHERICHIA COLI* SURVIVAL[1]

M. Kunzmann[a], L. Fermanyan[a], S. Tatikyan[b], G. Khachatryan[a].
[a]A.I.Alikhanyan National Science Laboratory (Yerevan Physics Institute), (Yerevan, Armenia),
[b]Candle Synchrotron Research Institute (Yerevan, Armenia).

Abstract. The essay represents the study of ultrashort electron beams impact on some *Escherichia coli K-12* strains with different radiosensitivity. The charged particle beams generated by ultrashort bunch accelerators differ by short duration of particle direct exposure, relatively long intervals between bunches and by high values of instantaneous dose rates. Because of these characteristics, the nature of ultrashort beams impact on biological objects may sufficiently differ from conventional sources of radiation. As a source of ultrashort beams linear electron accelerator AREAL of Cyclotron Research Institute CANDLE (Yerevan Armenia) was used. The dependence of *E.coli* cells survival from electron bunches repetition rate and irradiation media was investigated.
It is shown that the dose dependence of the survival degree of microorganisms has a qualitatively different unusual "concave" shape. Such behavior of the survival curves does not depend on the ionic composition of the irradiation medium as well as on the time of preliminary incubation of microorganisms in this medium.
To explain the observed phenomenon, it was assumed that the compensatory capacity of the irradiated object increases with an increase of the irradiation dose. The proposed mathematical model described well the behavior of the survival curves. It is assumed that this change of the compensatory capacity may be determined by oxygen.

Introduction. Currently, new technologies are intensively being developed in accelerator technology based on the application of ultrashort high frequency lasers. Such accelerators have femtosecond beams and high instantaneous dose values. One of these is AREAL electron accelerator, developed and constructed at CANDLE Institute with up to 5 MeV beam energy. 400 fs pulse duration and 2-50 Hz frequency (Tsakanov et al., 2016). Ultra-high dose rates of the electron beam are more effective when some cancer cell lines are irradiated. It may be expected that the application of ultrashort beams may lead to qualitatively new results and may be applied in radiotherapy, biotechnology and other fields. The main aim of this work is to carry out a comparative investigation of the effect of conventional and ultrashort electron beams on microorganisms.

---

[1] Theses of the article *Khachatryan G.E., Mkrtchyan N.I., Tatikyan S.Sh., Arakelyan V.B., Grigoryan B.A., Tsakanov V.M.* Affect of ultrashort electron beams on the *Escherichia coli* survival. Int J Adv Res 9(4), 2021, p. 211-217. http://dx.doi.org/10.21474/IJAR01/12675;
DOI:10.21474/IJAR01/12675

Materials and Methods. The research was carried out on E.coliK-12 strains AB 1157 - wild type. BL 1114 - radioresistant. AB 2463 – radiosensitive (Verbenko VN, 1994, Verbenko VN et al., 2000). For the cultivation of tested bacteria, a standard nutrient medium - Meat Peptone Agar (MPA) was used. All used chemicals were of analytical grade or higher except the technical agar-agar (Japan) that was carefully washed with a large amount of distilled water. The cell suspension for irradiation was obtained by inoculation of 5 ml of sterile irradiation medium with cell material from the colonies grown on Petri dishes, containing MPA. within 18-24 hours. As irradiation media 50 mM Potassum-Phosphate buffer with pH 7.0 (KPh) and low-ion drink water (DW) were used. The strains were irradiated on AREAL (CANDLE Synchrotron Research Institute). During the experiments the electron beam energy was 3.5 MeV. Pulse duration was 0.4 ps. The pulse frequency was 12 Hz. Average doze rate were 11.54 Gy/min. All the procedures were held at 20 °C. Right after the irradiation, cell suspension was diluted to concentrations convenient for further counting of the grown colonies, inoculated on MPA dishes and incubated in a thermostat, at 37°C during 24-36 hours. For counting, the dishes containing from 100 to 500 grown colonies were selected. The mathematical processing of the results was done by Wolfram Mathematica v ll.l.

Results and Discussion. In the first series of experiments the cell suspensions were irradiated by a beam with bunch frequency of 12 Hz in both irradiation media - KPh and DW. In Figure 1 the dependence of various E.coli strains survival on dose is shown. Hereinafter each point represents the average value of three independent experiments. As is seen from the picture, the form of the curves is different from a classical one and representing an L-shape form. With a change of irradiation medium salt composition some change in the value of D_{10} was observed, but this did not affect the character of the curves obtained.

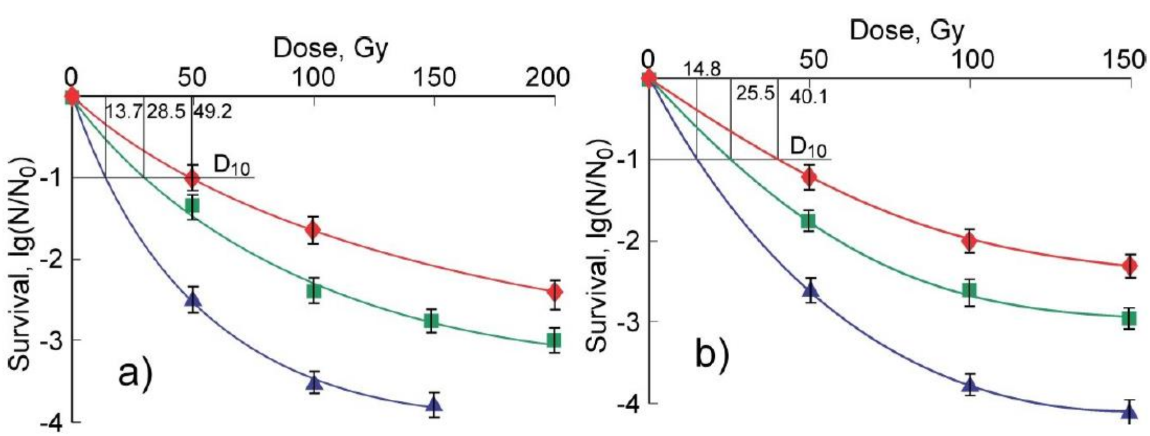

Figure 1. The dependence of E.coli strains survival on the dose value when irradiated with a beam of 12 Hz in DW (a) and in KPh (b). Triangles - radiosensitive (AB 2463), square - wild type (AB 1157) and diamonds - radioresistant (BL 1114).

The essential difference of AREAL from conventional sources of irradiation is that it generates ultrashort pulses of electron bunches of sub-picosecond duration. Such curves can be obtained if it is assumed that the compensating capacity of cells increases with increasing dose (D). The effect of compensatory capacity on survival can be taken into account if it is assumed that the reactivity R (D) is exponentially dose-dependent. "Compensatory ability" can be any physiological reaction of the body or even a physicochemical process that leads to a change in radiosensitivity upon exposure. According to the article (Hug and Kellerer, 1969; Dertinger and Jung, 1973), the following expression for survival was obtained, which took into account the increase in compensatory capacity with increasing radiation dose:

$$\ln(\mathrm{N}/\mathrm{N}_0) = -R_0 D - \frac{R_1}{\gamma}\left(1 - \exp(-\gamma D)\right) \tag{1}$$

where γ is the parameter, indicating on how fast the reactivity decreases from value $R_0 + R_1$ to R_0 with the increasing of irradiation dose. The curves in Fig. 1 were drawn according to formula (1) by the least squares method. As can be seen from Fig. 1, the theoretical dose dependence of survival (1) describes the experimental data quite well. It is of interest to compare the data obtained with the data from our earlier publication (Avakyan et al., 2011), which were obtained by irradiating the same deformations on a setup based on the MK-7.5 Microtron (electron beam energy 7.5 MeV, usual “quasi-continuous" beam, Petrosyan, 2009). The data from the article is shown in Fig. 2.

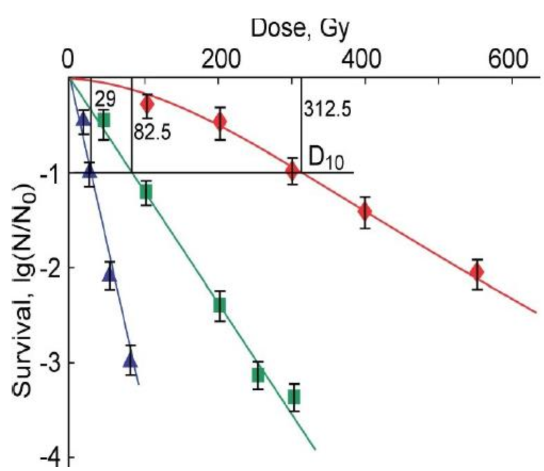

Figure 2. Irradiation of these *E.coli* strains using Microtron MK-7.5 with qiiasicontinious electron beam. Legends are the same as in figure 1.

In (Manti et al., 2017) a similar picture is cited when cells are irradiated with a pulsed beam (Dewey and Boag, 1959), which according to the authors is due to oxygen starvation - as a result of the rapid (because of large instantaneous dose) oxygen consumption by irradiated cells. The diffusion does not manage to entirely recover the oxygen level. As a rule, the absence of oxygen during cell irradiation results in increase of radioresistancy.

According to (Shinohara et al., 2004) the short time of beam direct effect on the sample, as well as the relatively long periods between the pulses result in a better neutralization of radicals through recombination, which in its turn leads to an increase in radioresistance.

Conclusion. It is shown that a qualitatively different unusual L-sliape dependence of microorganisms' survival degree on the dose is noted. Such shape of survival curves don't depends on ionic composition of the irradiation media.The increase of pulse frequency of electron bunches results in a noticeable increase of cells survival level.The positive reactivation is observed, that can be due to the oxygen.

References:

[1] Tsakanov VM, Amatuni GA, Amirkhanyan ZG, Aslyan LV, Avagyan VSh, Danielyan VA, Davtyan HD, Dekhtiarov VS, Gevorgyan KL, Ghazaryan NG et al. 2016. AREAL Test Facility for Advanced Accelerator and Radiation Source Concepts. Nuclear Instruments and Methods in Physics Research A, 829(1): 284-290.

[2] Verbenko VN, 1994. [Mutant alleles for radioresistance form the Escherichia coli strain Gam(r)444): cloning and preliminary characteristics]. (Russian), Genetika, 30(6), 756-762.

[3] Verbenko VN, Kalinin VL., Smolnikova AV. 2000. Effect of a null mutation In the PriA gene on the resistance of Escherichia coli. Genetika, 2000, 36(3), 315-321

[4] Hug O, Kellerer AM. 1969 Stochastic radiobiology. Moscow, Atomizdat 184 p. (in Russian).

[5] Dertinger H, Jung H. 1973. Molecular radiation biology – the action of ionizing radiation on elementary biological objects. Moscow, Atomizdat: 248p. (in Russian).

[6] Avakyan TsM, Mkrtchyan NI, Simonyan NV, Khachatryan GE. 2011. The research of biological action of the electrons with the energy of 7.5 MeV on the cells of *E.coli* K-12 bacteria having different reparation genotype. NAS RA Reports 111(2):164-170 (in Russian).

[7] Petrosyan ML, 2009. Interaction of accelerated electron beams with high-frequency electromagnetic fields. Abstract of Thesis for the degree of Doctor of Physical and Mathematical Sciences, https://www1.yerphi.am/PHD_Theses/doct_diss/Marzik-Avtoreferat6.pdf (in Russian).

[8] Manti L., Perozziello F.M., Borghesi M., Candiano G. et al. The radiobiology of laser-driven particle beams: focus on sub-lethal responses of normal human cells. http://iopscience.iop.org/1748-0221/12/03/C03084.

[9] Dewey DL and Boag JW 1959. Modification of oxygen effect when bacteria are given large doses of radiation. Nature 183:1450.

[10] Shinohara K, Nakano H, Miyazaki N, Tago M. and Kodama R. 2004. Effects of Single-pulse (< 1 ps) X-rays from Laser-produced Plasmas on Mammalian Cells. J. Radiat. Res. 45: 509-514.