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Effect of gamma-ray irradiation on *Escherichia coli* motility

Research Article

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Abstract: The effects of ionizing radiation on bacteria are generally evaluated from the dose-dependent survival ratio, which is determined by colony-forming ability and mutation rate. The mutagenic damage to cellular DNA induced by radiation has been extensively investigated; however, the effects of irradiation on the cellular machinery *in situ* remain unclear. In the present work, we irradiated *Escherichia coli* cells in liquid media with gamma rays from ^{60}Co (in doses up to 8 kGy). The swimming speeds of the cells were measured using a microscope. We found that the swimming speed was unaltered in cells irradiated with a lethal dose of gamma rays. However, the fraction of motile cells decreased in a dose-dependent manner. Similar results were observed when protein synthesis was inhibited by treatment with kanamycin. Evaluation of bacterial swimming speed and the motile fraction after irradiation revealed that some *E. coli* cells without the potential of cell growth and division remained motile for several hours after irradiation.

Keywords: *Escherichia coli* • *Flagellar motor* • *Gamma rays* • *Ionizing radiation* • *Motility*

1. Introduction

The effects of ionizing radiation on bacteria are often investigated within the context of meat sterilization. Generally, the effects are evaluated from the dose-dependent survival ratio, which is determined by colony-forming ability (usually measured as CFU, colony-forming unit) and mutation rate. Schilling *et al.* [1] reported that a 2 kGy dose of 10 MeV electron beam and X-ray irradiation effectively eliminated *Escherichia coli* O157:H7 in ground beef patties (from 10^3 CFU/g to less than 1 CFU/25 g) without affecting consumer acceptability (no change in appearance, texture, or flavor after irradiation). The D_{10} values for *E. coli* DSM498 after irradiation with a 10 MeV electron beam, estimated from survival curves, vary with the culture medium (D_{10} = 0.27 kGy in nutrient broth and D_{10} = 0.72 kGy in frozen meat) [2], probably because bacteria and food components, such as proteins, compete for interaction with radicals formed during the process [3]. Jeong *et al.* [4] have found that the low-energy X-ray irradiation sterilizes *E. coli* O157:H7 inoculated to iceberg lettuce at a dose less than gamma rays. More recently, the results of gamma radiation inactivation of *E. coli* in foods have been reported, showing that the D_{10} values for O157:H7 serotype and non-O157:H7 serovars were within the rage of 0.42 kGy – 0.52 kGy and 0.34 kGy – 0.41 kGy, respectively, while they varied with foods to which bacteria were inoculated (C. Sommers, O. J. Scullen and C.-A. Hwang, presented at Annual Meeting of International Association for Food Protection, Charlotte, NC, 28 – 31 July, 2013).

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Radiation-induced mutagenic damage to cellular DNA has been investigated extensively [5]. However, most methodologies estimate the degree of DNA damage, which inhibits cell growth, in specimens after bulk irradiation. The effects of irradiation on the cellular machinery *in situ* remain unclear. Recently, Ikeda *et al.* [6] developed an instrument that produces accelerated, micrometer-sized ion beams for the irradiation of biological materials in liquids. In a previous study, we irradiated single *E. coli* cells in liquid culture medium with a micrometer-sized proton beam to evaluate the effect of irradiation on cellular physiology. We found that the flagellar motor of *E. coli* stopped after bombardment with accelerated protons (H^+ beam at 2 MeV) at an average fluence of $2 \times 10^{12}/cm^2$, which corresponds to a dose of approximately 60 kGy [7]. In practice, bacterial cells become inert after exposure to a much lower dose of ionizing irradiation. In the present study, we evaluated the effect of gamma rays on the motility of *E. coli* cells. Our results showed that irradiation decreased the ratio of motile cells, but did not affect the swimming speed.

2. Experimental Procedures

2.1 Bacterial strain and culture media

The *E. coli* strain AW405 [8,9] used in this study was a gift from Dr. T. Minamino of Osaka University. L-broth contained 10 g of polypeptone (Nippon Seiyaku, Tokyo), 5 g of yeast extract (BD Japan, Tokyo), and 5 g of NaCl per liter. T-broth contained 10 g of polypeptone and 5 g of NaCl per liter. Cells were cultured in T-broth to observe flagellar motor activity and on L-agar plates (L-broth supplemented with 1% agar) to assess growth and division.

2.2 Gamma-ray irradiation and treatment with kanamycin

An aliquot (0.1 ml) of an overnight culture in T-broth was inoculated to 5 ml of fresh T-broth and incubated with shaking at 37°C for 3 h. At this point, cells were expected to be in log phase (active growth phase). Subsequently, the bacterial cultures were exposed to gamma rays from ^{60}Co in a water pool (~room temperature) for the period required to administer the designated dose. Because it took nearly 2 h to administer 8 kGy of irradiation, the first sampling time (defined as time = 0) was 2 h after the start of irradiation (Figure 1). Kanamycin was added to the cultures at a concentration of 25 mg mL^{-1}, and the first sampling time (defined as time = 0) was 2 h after the addition of kanamycin.

2.3 Examination of colony-forming ability

After irradiation with gamma rays, colony-forming ability was examined. Bacterial cultures irradiated with gamma rays (no irradiation control, 2 kGy, 4 kGy, 6 kGy, and 8 kGy) were serially diluted. Aliquots (0.1 ml) of the dilutions were spread on L-agar plates. In addition, an aliquot (0.1 ml) of a non-irradiated culture was spread on an L-agar plate containing kanamycin (25 mg mL^{-1}). Bacterial colonies were counted after overnight incubation at 37°C.

2.4 Examination of swimming speed

Bacterial cultures in T-broth were sampled at various time points and diluted 20-fold with 0.9% (w/v) NaCl. The diluted bacterial suspension was deposited in a 90-mm-deep well on a slide glass and covered with a cover glass. Bacterial swimming was photographed over 1-sec exposures using an Axiostar microscope (Zeiss, Oberkochen) equipped with a Coolpix 4500 digital camera (Nikon, Tokyo). The tracks of swimming bacteria were measured using ImageJ software ver. 1.44p (National Institutes of Health, http://rsb.info.nih.gov/ij/). The fraction of motile cells was estimated by dividing the number of swimming cells (tracks) by the total number of cells observed in the microscopic field.

2.5 Estimation of molecular size of target proteins

The sensitivity of an organism to irradiation is generally described by the D_{10} value, the radiation dose that reduces activity to 10% (1/10). When evaluating the

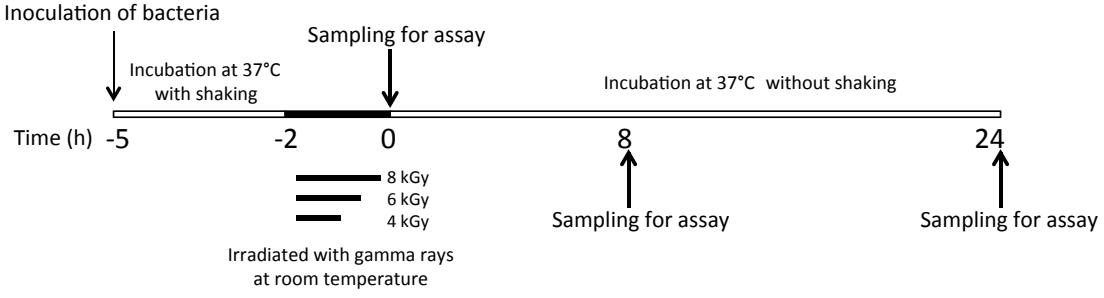

Figure 1. Scheme for sampling. First sampling time (the end of irradiation treatment) was defined as time = 0. Open lines indicate incubation at 37°C, and the solid line indicates incubation at room temperature.

inactivation of proteins and chemicals, the D_{37} value, the dose that reduces activity to 37% (= 1/*e*, where *e* is Napier's constant), is often used instead. When activity decreases exponentially with dose, D_{10} values can be converted to D_{37} values using the following formula: $D_{37}/D_{10} = \log_{10}e^{-1}/\log_{10}10^{-1} = 0.434$. According to the literature [10-13], the functional molecular size (Mr) can be estimated from the activity that remains after irradiation, using the formula $Mr = (6.4 \times 10^6)/D_{37}$, where D_{37} is presented in kGy.

3. Results

3.1 Colony-forming ability after gamma-ray irradiation or treatment with kanamycin

The effects of gamma-ray irradiation and the effects of kanamycin were first assessed by measuring colony-forming ability on L-agar plates. The results are shown in Figure 2a (closed circles). When 0.1 ml of sample cultures irradiated with 6 kGy or 8 kGy was deposited on L-agar plates, no colonies appeared after an overnight incubation. In addition, no colonies appeared on L-agar plates containing kanamycin. Assuming that the dose response to irradiation approximated a single exponential decline, we calculated a D_{10} value of 0.56 kGy, and a D_{37} value of 0.24 kGy. The results presented here are consistent with the known D_{10} values for *E. coli*, reported as 0.23 – 0.47 kGy [2,14]. Extrapolation of the results suggested that the colony-forming ability reached 10^{-12} at a dose of 6.7 kGy.

3.2 Effect of gamma rays or kanamycin on bacterial swimming

Representative images of swimming bacteria are shown in Figure 3. With irradiation, the fraction of motile cells at the first time point (time = 0, defined in Figure 1) decreased in a dose-dependent manner (Figure 2a, closed triangles). On the other hand, irradiation appeared to have little effect on the swimming speed of the motile cells (Figure 2b, closed boxes).

After further incubation (time = 8, and 24 h), the motile fraction of the cells tended to decrease (Table 1). In the cells which were not irradiated but incubated in the presence of kanamycin, the motile fraction also

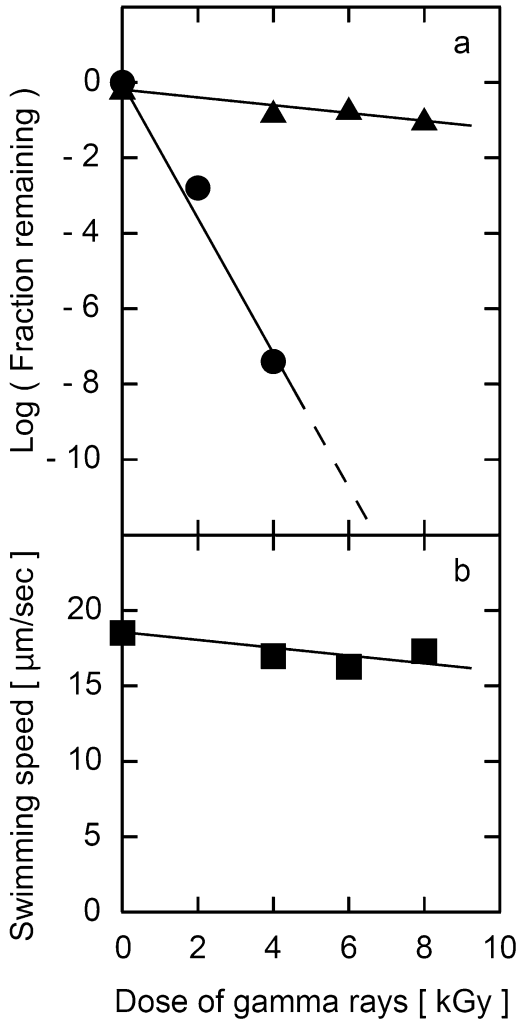

Figure 2. Sensitivity of *E. coli* strain AW405 to gamma-ray irradiation from ^{60}Co in liquid media. (a) Colony forming ability (●) and motile fraction (▲) at time = 0; (b) swimming speed of the motile fraction (■) at time = 0.

	Ratio of swimming cells to total cells in the field				
Time (h)	0 kGy	4 kGy	6 kGy	8 kGy	Kanamycin
0	0.70	0.17	0.20	0.11	0.17
8	0.48	0.15	0.24	0.03	0.10
24*	0.17	0.09	0.09	N.D.	N.D.

Table 1. Changes in the motile fraction after irradiation or treatment with kanamycin.

**For the kanamycin experiment, the sampling time was 26 h. N.D., not determined because less than one swimming cell on average was observed in the field.*

decreased (Table 1). Non-motile cells seemed to adhere to the glass surface rather than drifting in the well (Figure 3). As for the swimming speed, neither irradiation nor kanamycin treatment had any apparent effect on the swimming speed of the motile cells, which decreased gradually over time (Table 2). As the results, the motile fraction decreased in time course and in dose-dependent manner, but the swimming speed of motile cells remained nearly identical regardless with irradiation dose.

Cells of various sizes were observed in irradiated samples (Figure 3b) and kanamycin-treated samples (Figure 3c) because the treatments stopped cell growth at log phase. Thus, these samples contained cells at various stages of growth, including newly divided cells (small), elongating cells (moderate), cells ready to divide (long), and cells that failed to divide (very long). On the other hand, smaller cells were present in control samples (Figure 3a). Cells in the control samples were viable, but were unable to elongate after division because of environmental limitations, such as nutrient depletion during incubation. The variations in cell size may have introduced errors when measuring swimming speed. However, the size variation was not so large (mostly within a range of approximately 2 μm) relative to the track lengths, and the measurements in the present study were sufficient for evaluating the effects of irradiation.

4. Discussion

In peritrichously flagellated *E. coli*, flagellar filaments (usually about 4 on average) form a bundle through the coordinated rotation of their flagellar motors, which are found at the base of each filament. Thus, bacterial swimming reflects the action of all of the motors [15]. Currently, it is not known how the presence of active and inactive motors in a single cell affects the swimming behavior of bacterial cells. According to a well-accepted model for motor construction [15-20], each flagellar motor in *E. coli* has about 8 ~ 11 force-generating units composed of MotAB complexes, which are thought to be the reaction center of the molecular motor. These force-generating units may work independently and additively. The effect of inactive unit(s) on motor activity has not yet been established [21]. In addition, the activity of the flagellar motors is affected by many other factors, such as the activity of respiratory chains, which maintain the electrochemical potential of protons across the membrane. Furthermore, little is known about the effect of gamma rays on the ability of bacteria to adhere to a glass substrate. Under these circumstances, it is difficult to explain why and how gamma-ray irradiation decreases the motile fraction without affecting the swimming speed.

A dose-dependent effect of gamma-ray irradiation on the swimming speed of motile cells was not

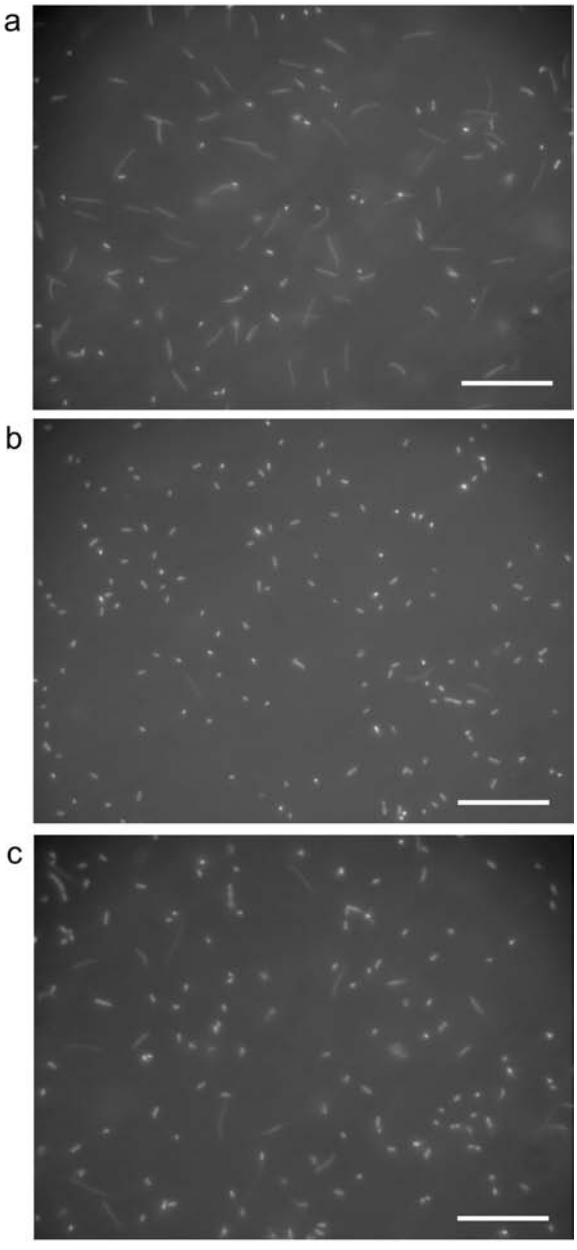

Figure 3. Swimming bacteria after 8 h of treatment. (a) A representative image of non-irradiated control cells sampled at 8 h; (b) a representative image of cells irradiated with gamma rays at a dose of 8 kGy sampled at 8 h; (c) a representative image of cells incubated in the presence of kanamycin sampled at 8 h. Pictures were taken in dark-field mode with an exposure time of 1 sec. Swimming cells appear as tracks, adherent cells appear as bright spots, and drifting or tethered cells appear as light blobs. The scale bar (50 μm) is shown at the bottom right.

Time (h)	Swimming speed ± s.d. (mm/sec) Coefficient of variation (n: data number) 0 kGy	4 kGy	6 kGy	8 kGy	Kanamycin
0	18.5±4.3 0.23 (n = 1112)	17.0±4.2 0.25 (n = 589)	16.3±4.1 0.25 (n = 1250)	17.3±4.0 0.23 (n = 358)	18.1±4.3 0.24 (n = 212)
8	13.1±3.6 0.28 (n = 1067)	14.4±3.6 0.25 (n = 570)	14.3±3.3 0.23 (n = 370)	13.3±3.1 0.23 (n = 109)	14.8±3.9 0.26 (n = 139)
24*	10.6±3.1 0.29 (n = 287)	12.7±3.3 0.26 (n = 88)	12.0±2.9 0.25 (n = 107)	12.0±2.6 0.22 (n = 30)	11.6±3.1 0.27 (n = 37)

Table 2. Changes in swimming speed after irradiation or treatment with kanamycin.

**For the kanamycin experiment, the sampling time was 26 h.*

observed. This might suggest a one-hit inactivation model for bacterial swimming. Our previous studies of the motion of a single flagellar motor bombarded with accelerated protons suggested that the rotation of the motor became irregular after the first hit and stopped after some additional hits [7]. The first hit to a motor was more likely to have a strong effect on the swimming cells. These phenomena may reflect the radiation-induced inactivation of the proteins that are responsible for activity of flagellar motors. In the present study, by fitting the motile fraction data at time = 0 to a single exponential, we calculated a D_{10} value of 9.4 kGy and a D_{37} value of 4.1 kGy. The D_{37} value corresponds to a protein of 1560 kDa, according to the radiation inactivation assays (see Experimental Procedures). This molecular size of 1560 kDa was comparable to 8 times of a MotAB complex, that consists of four MotA and two MotB subunits [15,20], in good agreement with the number of force-generating units in a flagellar motor. Of course there are many other proteins (or protein complexes) that are indispensable for bacterial motility; *e.g.*, other components of the motor, respiratory chains to maintain the electrochemical potential of protons across the membrane, and any other proteins in metabolic pathways. These proteins should be damaged when treated with ionizing radiation. It might also be plausible that a part of cell membrane was damaged to collapse the electrochemical potential across the membrane. Membrane damages have been shown to induce the release of membrane-associated proteins and cytoplasmic enzymes such as lactate dehydrogenase (LDH) in human erythroleukemic cells [22]. It may cause changes in energy metabolisms in the affected cells. This idea may be supported by the results that the *E. coli* transformant producing exogenous OsLEA5 protein, that confers stabilization of LDH, exhibited improved resistance against diverse abiotic stresses: high salinity, osmotic, freezing, heat, and UV radiation [23].

Another factor to decrease the motile fraction of irradiated cells may be changes in ability of the cells to adhere to the glass substrate. In the early stage of biofilm formation, bacterial cells slow their swimming speed to attach the surface of substrate [20]. In our observations, we did not see any slowed-down swimmers, but there is another possible explanation of adhesion of cells to the glass substrate, that the cells, having damages in their cell membrane, became more adhesive to the glass.

The effects of kanamycin, an antibiotic that inhibits prokaryotic protein synthesis, on motility were similar to those of 8-kGy irradiation (Table 1 and Table 2). After kanamycin treatment, the motile fraction decreased over time, but the swimming speed appeared unchanged, when compared to that of control samples. Ionizing radiation damages DNA and proteins. Gene expression ceases in heavily damaged cells, as it does in cells treated with kanamycin. Turnover of some components of the flagellar motor has been reported [20]. This may mean that new protein synthesis is required to maintain the activity of the flagellar motor machinery.

References

[1] Schilling M.W., Yoon Y., Tokarskyy O., Pham A.J., Williams R.C., Marshall D.L., 2009, Effects of ionizing radiation and hydrostatic pressure on Escherichia coli O157:H7 inactivation, chemical composition, and sensory acceptability of ground beef patties, Meat Science 81, 705-710

[2] Mayer-Miebach E., Stahl M.R., Eschrig U., Deniaud L., Ehlermann D.A.E., Schuchmann H.P., 2005, Inactivation of a non-pathogenic strain of E. coli by ionising radiation, Food Control 16, 701-705

[3] Patterson M., 1989, Sensitivity of Listeria monocytogenes to irradiation on poultry meat and

in phosphate-buffered saline, Lett. Appl. Microbiol. 8, 181-184
[4] Jeong S., Marks B.P., Ryster E.T., Moosekian S.R., 2010, Inactivation of Escherichia coli O157:H7 on lettuce, using low-energy X-ray irradiation, J. Food Protect. 73, 547-551
[5] Cadet J., Douki T., Gasparutto D., Ravanat J-L., 2005, Radiation-induced damage to cellular DNA: measurement and biological role, Radiat. Phys. Chem. 72, 293-299
[6] Ikeda T., Kanai Y., Iwai Y., Kojima T. M., Maeshima K., Meissl W. et al., 2011, Glass capillary optics for producing nanometer sized beams and its applications., Surf. Coat. Technol. 206, 859-863
[7] Kato M., Meissl W., Umezawa K., Ikeda T., Yamazaki Y., 2012, Real-time observation of Escherichia coli cells under irradiation with a 2-MeV H^+ microbeam, Appl. Phys. Lett. 100, 193702
[8] Armstrong J. B., Adler J., Dahl M.M., 1967, Nonchemotactic mutants of Escherichia coli, J. Bacteriol. 93, 390-398
[9] Minamino T., Imae Y., Oosawa F., Kobayashi Y., Oosawa K., 2003, Effect of intracellular pH on rotational speed of bacterial flagellar motors, J. Bacteriol. 185, 1190-1194
[10] Kepner G.R., Macey R.I., 1968, Membrane enzyme systems. Molecular size determinations by radiation inactivation, Biochim. Biophys. Acta 163, 188-203
[11] Kempner E.S., Schlegel W., 1979, Size determination of enzymes by radiation inactivation, Anal. Biochem. 92, 2-10
[12] Nugent J.H., 1986, Molecular-size standards for use in radiation-inactivation studies on proteins, Biochem. J. 239, 459-462
[13] Kempner E.S., 1999, Advances in radiation target analysis, Anal. Biochem. 276, 113-123
[14] Mahapatra A.K., Muthukumarappan K., Julson J.L., 2005, Applications of ozone, bacteriocins and irradiation in food processing, a review, Crit. Rev. Food Sci. Nutr. 45, 447-461
[15] Berg H.C., 2003, The rotary motor of bacterial flagella, Annu. Rev. Biochem. 72,19-54
[16] Block S.M., Berg H.C., 1984, Successive incorporation of force-generating units in the bacterial rotary motor, Nature 309, 470-472
[17] Blair D.F., Berg H.C., 1988, Restoration of torque in defective flagellar motors, Science 242, 1678-1681
[18] Muramoto K., Sugiyama S., Cragoe E.J. Jr., Imae, Y., 1994, Successive inactivation of the force-generating units of sodium-driven bacterial flagellar motors by a photoreactive amiloride analog, J. Biol. Chem. 269, 3374-3380
[19] Reid S.W., Leake M.C., Chandler J.H., Lo C.-J., Armitage J.P., Berry R.M., 2006, The maximum number of torque-generating units in the flagellar motor of Escherichia coli is at least 11, Proc. Natl. Acad. Sci. USA 103, 8066-8071
[20] Brown M.T., Delalez N.J., Armitage J.P., 2011, Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor, Curr. Opin. Microbiol. 14, 734-740
[21] Atsumi T., 2001, An ultrasonic motor model for bacterial flagellar motors, J. Theor. Biol. 213, 31-51
[22] Jurisić V., Spuzić I., Konjević G. 1999, A comparison of the NK cell cytotoxicity with effects of TNF-alpha against K-562 cells, determined by LDH release assay, Cancer Lett. 138, 67-72
[23] He S., Tan L., Hu Z., Chen G., Wang G., Hu T., 2012, Molecular characterization and functional analysis by heterologous expression in E. coli under diverse abiotic stresses for OsLEA5, the atypical hydrophobic LEA protein from Oryza sativa L, Mol. Genet. Genomics 287, 39-54