



# Radio Sensitization By Static Electric Fields Is Observed In The Extremophilic Deinococcus Radiodurans Exposed To Gamma Radiation

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**Submitted on:**18-Jun-2014, 02:13:49 PM GMT

**Accepted on:**06-Jul-2014, 08:13:16 AM GMT

**Article ID:** WMCPLS00385

**Article Type:** Research articles

**Article URL:** [http://webmedcentralplus.com/article\\_view/385](http://webmedcentralplus.com/article_view/385)

**Subject Categories:**BIOPHYSICS

**Keywords:**Deinococcus radiodurans; gamma radiation; electric field; cell viability decrease; repairing shoulder depletion

**How to cite the article:**

Arruda-Neto JD, Segreto HR, Gomez JG, Silva LF, Jorge SA, Mendonca TT, Nieto L , Cavalcante GT, Rodrigues TE, Righi H , Prado GR, Genofre GC. Radio Sensitization By Static Electric Fields Is Observed In The Extremophilic Deinococcus Radiodurans Exposed To Gamma Radiation. *WebmedCentral plus* BIOPHYSICS 1970;-39(1):WMCPLS00385

**Source(s) of Funding:**

None

**Competing Interests:**

None

# Radio Sensitization By Static Electric Fields Is Observed In The Extremophilic *Deinococcus Radiodurans* Exposed To Gamma Radiation

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## Abstract

**Background** – *Deinococcus radiodurans* is one of the fiercest radioresistant organisms, exhibiting sophisticated mechanisms for both proteome radiation protection and DNA repair responsible for its extremophilic character. It would be a quite important task the challenging of *D. radiodurans* formidable viability performance when simultaneously exposed to gamma radiation and a newly observed radiation sensitizer, Static Electric Field (SEF), *vis-à-vis* more radiosensitive organisms previously studied at this Laboratory.

**Methods** – Cultures of this organism were harvested during the exponential and stationary growth phases and irradiations were performed with a  $^{60}\text{Co}$  gamma source facility in the dose interval 0 – 12 kGy. Immediately after irradiation the cells were exposed to a  $2 \text{ kV}\cdot\text{cm}^{-1}$  SEF for 10 hours and the number of colonies was counted after a 36-hour period of incubation.

**Results** – An intriguing and significant depletion of the repairing shoulder, from 8 kGy to 4 kGy, was found when *D. radiodurans* is exposed to the electric field subsequent to irradiation. Furthermore, analysis of survival curves shows that at doses equal and higher than 4 kGy a mere additional dose of 0.9 kGy kills off 63% of the cells. These findings are tentatively addressed in terms of a biophysical *ad-hoc* approach.

**Conclusions** – It is concluded that SEFs are highly efficient radio-sensitizers, a finding that could be explored for therapy purposes. These first time and intriguing conclusions suggest *cum grano salis* that SEFs scramble reassembling of small, high-dose produced, DNA fragments, therefore preventing efficacious repair processes.

**Keywords:** *Deinococcus radiodurans*; gamma radiation; electric field; cell viability decrease; repairing shoulder depletion

## Introduction

Preponderance of evidences suggests that stand-alone effects on biological systems by exposure to alternating electric and magnetic fields do not have genotoxic potential, as reviewed and discussed elsewhere [1]. Low-intensity, alternating electric fields on the other hand, was found to have an inhibitory effect on the growth rate of malignant tumors in animals [2]. As experimentally shown in this and previous works carried out at this Laboratory, intense Static Electric Fields (SEF) also have no genotoxic potential in eukaryotes and prokaryotes cells [3], [4]. The effects on humans after exposure to high Static Electromagnetic Fields (up to 9.4 tesla) were intensively and extensively reviewed by Yamagichi-Sekino et al. [5].

Another quite recent study examined the effects of electromagnetic fields (EMF) combined with at least one other agent, as gamma radiation, heat, chemotherapeutic drugs, etc., on biological systems [6]. It is described the wide range of potential effects in which EMF play a supportive role. In this case, beneficial effects include improved treatment of chronic diseases like e.g. cancer, by enhancing ionizing radiation or chemotherapy, while adverse effects include enhanced carcinogenesis, cellular or genetic mutations, and teratogenicity. It was put in evidence that, although there is a substantial body of evidence supporting these findings yet there is, on the other hand, no consensus on their potential effects, either beneficial or adverse. [6].

As recently observed, however, the combined exposure of some moderately radioresistant cell lines to Static Electric Fields (SEFs) and ionizing radiation raises cell death, thus indicating that SEFs act as radiation sensitizers [3], [4]. For instance, the prokaryote *Microcystis panniformis* presents a repairing shoulder of approximately 2.5 kGy (and  $D_{37} = 3.7 \text{ kGy}$ ), but when simultaneously exposed to radiation and a SEF the shoulder decreases to 2.0 kGy (with  $D_{37} = 2.8 \text{ kGy}$ ) [3].  $D_{37}$  is the dose which yields a 37% survival, calculated on the repairing shoulder.

The putative biological role of SEFs as radiation sensitizers is an intriguing and relevant issue, since stand-alone exposure to this exogenous physical agent is not genotoxic. Such circumstance served as motivation to here examine the limits of this radio sensitization process by carrying out viability measurements with a very radioresistance cell line.

In this regard, *Deinococcus radiodurans* exhibits an extraordinary ability to withstand lethal and mutagenic effects of DNA damaging agents [7], [8]. It is the best-known extremophile among a handful of organisms found to resist extremely high exposures to desiccation and ionizing radiation [9]. Both desiccation and radiation cause extensive intracellular DNA double strand breakage (DSB). Standard vegetative prokaryotic and eukaryotic cells can repair less than a dozen simultaneous DSBs, while *D. radiodurans* survives ionizing radiation breaking its genome into several hundred fragments.

In fact, the 8 kGy wide *repairing shoulder* of *D. radiodurans* shows that the repair mechanism of this bacterium is highly proficient, therefore explaining its extremely high radioresistance. This would indicate that the survival of *D. radiodurans* and other strongly-radiation-resistant organisms is consequence of enhanced DNA repair.

However, this conceptual scenario in radiation biology is changing, as recently proposed by Krisko and Radman [9]. In fact, there has to be in *D. radiodurans* a regulatory interplay between different processes associated with oxidative stress response pathways [7], [10], [11]. It is advocated that the proteome, rather than the genome, is the prime target in radiation-induced cell death, since survival itself depends primarily on vital functions performed by the proteome [12-14]. In this sense, protein protection would be an important player contributing to the high efficiency of DNA repair enzymes of *D. radiodurans* [7], [15].

Actually, with the pool of repairing proteins preserved the cell is able to perform DNA repair more efficiently. At the edge of the process, viability is determined by successful repair of Double Strand Breaks (DSBs). A single DSB left unrepaired leads cell to death [16]. Regarding efficient DNA repair, Zahradka and collaborators [17] described a two-stage DNA repair process for *D. radiodurans*, involving a new molecular mechanism for reassembly of shattered chromosomes called *extended synthesis-dependent strand annealing* (ESDSA). All known mechanisms possibly rejoining hundreds of partially overlapping chromosomal fragments were ruled out, indicating that stress tolerance mechanisms in *D. radiodurans* differ from

established paradigms.

Although all the sophisticated peculiarities of *D. radiodurans* to cope with huge doses of gamma radiation, as protein protection plus ESDSA chromosomal fragments rejoining, it was demonstrated that this bacterium succumbs to irradiation with intense electron beams from a Linear Accelerator, as recently reported in this journal [18]. This intriguing finding could be put in evidence with the high tolerance of *D. radiodurans* to stresses as acute as energetic hadrons beams [19].

The main purpose of this study, therefore, is the challenging of *D. radiodurans* formidable viability performance, against gamma radiation exposure, by a newly observed radiation sensitizer [3], a Static Electric Field (SEF), *vis-à-vis* more radiosensitive organisms also previously studied in this Laboratory [3], [4].

It is noted, additionally, that many cell recovering mechanisms in bacteria are conserved in eukaryotes. Hence, a comparative study of their responses to combined ionizing radiation and SEFs exposure is both relevant and opportune. In fact, *D. radiodurans* is also rather inspiring for a number of radiobiological applications as e.g. the use of this microorganism as a bacterial model for both oncology [20] and the study of microbial ability to survive in space conditions, envisaging applications in Astrobiology [21].

## Materials and methods

Cultures of *Deinococcus radiodurans* R1, GY 9613 wild-type strain, were obtained from a stock kept at the Institute for Radioprotection and Dosimetry/(IRD/RJ), Rio de Janeiro, Brazil. The cells in freezer stocks (glycerol 10%, -80°C) were streaked in solid TGY medium (1% tryptone, 0.2% glucose, 0.6% yeast extract, 1.5% agar) (Oxoid LTD, Basingstoke, Hampshire, England) and incubated for 24 hours (30 ± 1°C). Ten colonies from this agar plate were used to inoculate liquid TGY medium and incubated for 24 hours in a rotary shaker (30 ± 1°C, 200 rpm) (model TE 420, from Tecnal, São Paulo, Brazil). The culture of *D. radiodurans* is axenic (pure) and as a consequence, monoclonal. Therefore, the collection of 10 colonies is only a standard procedure to inoculate a controlled amount of cells. A volume of this culture, corresponding to 10% of the final volume, was used to inoculate fresh liquid TGY medium and incubated in a rotary shaker (30 ± 1°C, 200 rpm). Samples of this culture were harvested during the exponential and stationary growth phases. Cells were pelleted by

centrifugation (10,000xg, 5 min., 5 °C), washed with physiological solution (NaCl 0.85%, pH equal to 6.8), re-suspended in the same volume of physiological solution and distributed in 2 mL aliquots in microcentrifuge tubes.

Irradiation of the cells was carried out with a  $^{60}\text{Co}$  gamma source facility (Gammabeam, model 650 from MSD Nordion, Ottawa, Canada), with doses in the interval 0 – 12 kGy at a dose rate of 2.4 kGy/h. The geometry of the Gammabeam  $^{60}\text{Co}$  elements is  $4\pi$  sr, allowing for homogeneous irradiation of the samples. The samples were exposed to a static electric field of  $2 \text{ kV}\cdot\text{cm}^{-1}$  between the plates of a capacitor for 10 hours, immediately after irradiation. This electric field was produced by a conventional DC high-voltage power supply operating from 0 to 10 kV, with its two poles connected to the aluminum parallel and circular plates (25cm radius) 2 cm from each other. Field strength is given by the applied voltage divided by 2cm. It is clear that  $2 \text{ kV}\cdot\text{cm}^{-1}$  is the *externally applied field*. However, the physiological solution in which the cells were suspended is mildly saline (NaCl 0.85% only), which reduces a little bit the *effective electric field* to which the cells are exposed.

Three sets of samples were prepared and submitted to three different control conditions. Control-set-A: was only exposed to static electric fields (SEF) for a period of 10 hours. Control-set-B: was incubated in the buffer after irradiation for the same amount of time as SEF exposure (10 hours). Control-set-C: constituted of samples neither exposed to radiation nor to SEF.

To evaluate the concentration of viable cells (CFU/mL – colony-forming units per milliliter), treated and untreated samples were submitted to serial decimal dilutions and plated on solid TGY medium. The number of colonies was counted after 36 hours of incubation ( $30 \pm 1^\circ\text{C}$ ). Optical cell density (OD) was measured at 600 nm (Cary 50 Bio UV Visible Spectrophotometer, from Varian, Mulgrave, Australia). Measuring OD values during cell growth is a rapid and currently used method to monitor cell growth phases in culture. The initial number of cells ( $D = 0 \text{ kGy}$ ) in all irradiations was maintained between  $1.0$  and  $1.4 \times 10^9$ .

The number of cells at the exponential phase (around 8h – see Figure 1) is much smaller than in the stationary phase. Because of this, we also performed irradiation of *D. radiodurans* in the exponential phase with cell number similar to the stationary phase ( $10^8$  cells/mL), but only at doses of 2, 4, 6 and 8 kGy for checking purposes. This adjustment of cell numbers in these two growing phases was performed by means of trial dilutions until obtaining approximately equal optical densities.

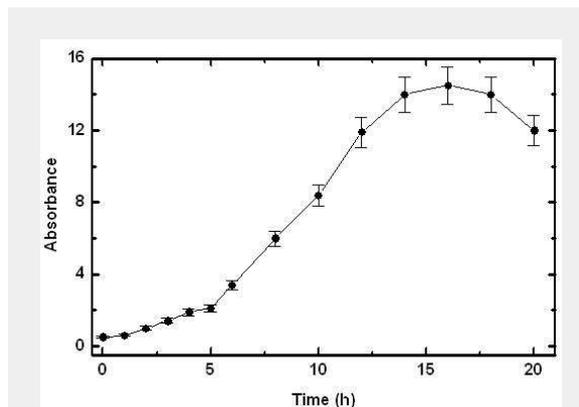


Figure 1: Growth curve of *D. radiodurans* control sample. The lines in this and in the other figures are only eye guidelines, that is, these lines are not the result of fitting procedures.

All exposures were performed at controlled temperature, continuously remote-monitored by a Digital Thermometer TH-1200 C model (INSTRUTHERM, USA), equipped with Chromel – Alumel sensors (HOMIS, USA). *D. radiodurans* sample temperatures were maintained at  $(6.5 \pm 0.5)^\circ\text{C}$ , within the optimum 5 – 10 °C temperature range to keep growth and metabolic processes minimized during exposure to radiation. It is wise to remember, *D. radiodurans* is resistant not only to radiation but to heat treatment too [20], and its optimum growth temperature is 30 °C.

All measurements were performed in triplicate for each of the 13 irradiation doses in the interval 0 – 12 kGy. Samples were collected from a single culture, but *three independent irradiations* were performed for each dose and *on different days*. In this sense, 39 independent data taking has been carried out. Each data point displayed in the figures corresponds to an average of the three independent irradiations.

In the experiments with exposure to gamma radiation and a Static Electric Field, triplicate measurements were carried out at 5 independent irradiations (15 data taking).

Thus, error bars showing up in figures represent external standard deviations. Data handling procedure consisted solely of data averaging; thus, only the external standard deviation of the averaged values was calculated, a simple and conventional parametric statistic in the normal model[22].

## Results

### 3.1 Control samples

Cells may die following exposure to a static electric

field (SEF) for 10 hours, presumably because of the lack of necessary nutrients required for protein synthesis and DNA repair, a valid concern in the present study. In this regard, the measured differences of colony number per plate from the control- set-B (see Material and Methods), between  $t = 0\text{h}$  and  $t = 10\text{h}$  (where  $t$  is the exposure time to the SEF) were, on average, smaller than 30. These differences are similar to errors inherent in dilution and plating. Likewise, differences of colony number between control- set-A and control- set-C (not exposed either to radiation or to SEF) were equally negligible, implying that sole exposure to non-pulsed electric fields is not cytotoxic.

### 3.2 Growth and survival curves

The growth curve of *D. radiodurans* obtained here is shown in Figure 1, exhibiting the characteristic exponential and early stationary growth phases while the corresponding survival curves,  $S(D)$ , are shown in Figure 2 as a function of doses ( $D$ ). The stationary phase exhibits the well-known 8 kGy wide *repairing shoulder*, meaning that in a dose-interval where  $S(D)$  slowly varies the cell recovering processes are efficient [23].

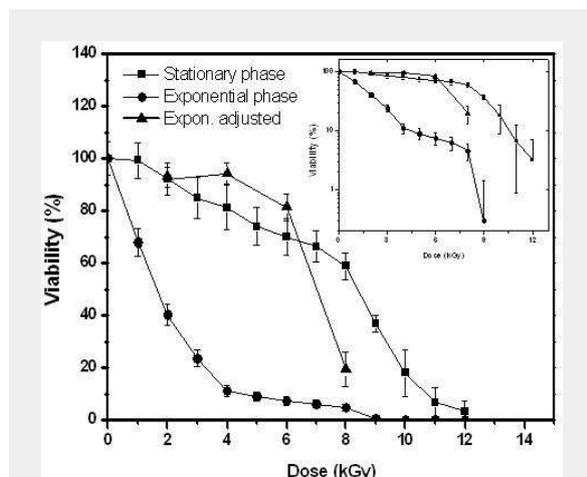


Figure 2: Survival curves of *D. radiodurans* after irradiation obtained with samples in the stationary growth phase (squares), and in the exponential phase with (triangles) and without (circles) number of initial cell adjustment (see text for details). Insert: the same in log-linear plot. ( $p < 0.001$ )

The *D. radiodurans* radiosensitivity in the exponential phase is quite dependent on cell concentration, but not in the stationary phase. Viability in the exponential phase with number of cell adjustment (see Material and Methods) is consistent with other findings [24], [25]. The survival curves following exposure to gamma radiation ( $\gamma$ ), with and without exposure to Static Electric Field (SEF) are shown in Figure 3. Survival

curves are expressed as percentage fractions of the corresponding control (*zero dose points* in Figures 2 and 3) – in this sense,  $S(0\text{ kGy}) = 100\%$ . The inserts of these figures show the data in log-linear scales, the standard for presenting radiobiological results.

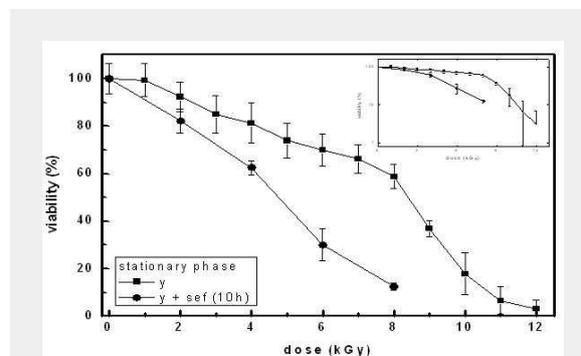


Figure 3A: Survival curves of *D. radiodurans* after irradiation ( $\gamma$ ) and after irradiation plus exposure to static electric field (SEF) of  $2\text{ kV}\cdot\text{cm}^{-1}$  by 10h. All samples were obtained during the stationary growth phase. Insert: the same in log-linear plot. ( $p < 0.001$ )

### 3.3 Statistical analysis

It is shown in this work that the viability (%) mean values measured with irradiation only, are statistically different from the mean values measured with irradiation plus exposure to a SEF (see survival results in Figure 3 with their standard deviations). This verification was carried out dose by dose as described below.

According to the Tukey test, two mean quantities are statistically different when the absolute value of the difference between them ( $\Delta M$ ) is always equal or greater than the minimal significant difference ( $msd$ ). The latter, on the other hand, is proportional to the square root of the ANOVA residual mean square ( $rms$ ), that is,  $msd \approx (rms)^{0.5}$ .

Plotted in Figure 4 is the absolute value difference between the surviving curves of *D. radiodurans* following exposure to radiation with and without exposure to the Electric Field ( $\Delta M$ ), plus the calculated curve for the minimal significant difference ( $msd$ ). A mere visual inspection of this figure led to the conclusion that the above-mentioned Tukey's criterion had been fulfilled[22], [26]. In fact,  $\Delta M$  is from 2 to 9 times higher than the  $msd$  in the 4 to 8 kGy dose range.

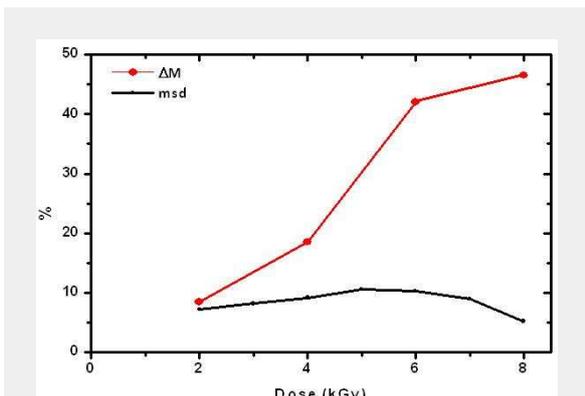


Figure 4: Data points ( $\Delta M$ ): absolute value differences between the surviving curves of *D. radiodurans* shown in Figure 4 ( $\gamma$  and  $\gamma + sef$ ). Curve (msd): minimal significant difference. (See text for details).

### 3.4 Survival curves slopes – loss of cellular viability

The particular data interpretation in this study requires quantification of the survival curve slopes (their first derivative), which is straightforwardly estimated from linear-linear plots. In this sense, also defined here is the *loss of cellular viability* as the first derivative of the survival curve,  $dS/dD$ .

The results from irradiations with gammas exhibit two distinct behaviors at the dose intervals 0 – 8 kGy and 8 – 10 kGy (Figure 2). If  $S(D)$  is fitted by straight lines in these two dose intervals we obtain  $dS/dD = -(5.0 \pm 0.5) \text{ kGy}^{-1}$  and  $dS/dD = -(20 \pm 1) \text{ kGy}^{-1}$ , respectively. Thus, in the interval 0 – 8 kGy known as the *repairing shoulder* the viability loss is approximately 5% per kGy, while at doses higher than 8 kGy it is nearly 20% per kGy.

In irradiations with gammas followed by exposure to the SEF (Figure 3) a *repairing shoulder* is no longer clearly observed. Instead, there is a pronounced decrease in viability. Again, by fitting straight lines to  $S(D)$  in the dose intervals 0–4 kGy and 4 – 8 kGy,  $dS/dD = -(9 \pm 1) \text{ kGy}^{-1}$  and  $dS/dD = -(14 \pm 2) \text{ kGy}^{-1}$  are obtained (Figure 3B), respectively, to be compared with the results for irradiation only (see Table 1). These observed depletions of the *repairing shoulder* are a consequence of a substantial reduction in cell viability, as statistically attested to in Figure 4.

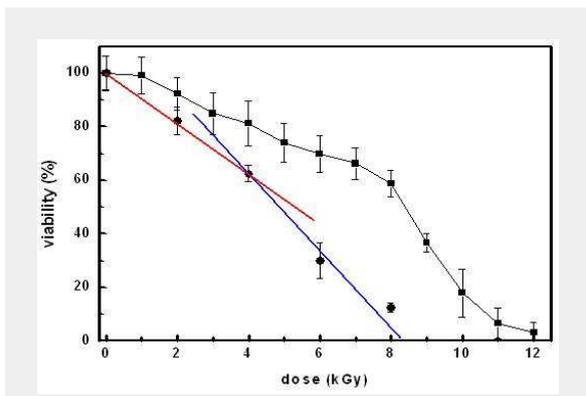


Figure 3B: Same curves as in (A) plus the straight lines fitted to the dose intervals 0 – 4 kGy and 4 – 8 kGy of the  $\gamma + SEF$  data set (details in the text).

| Dose-range<br>(kGy) | $\text{kGy}^{-1}$        |                                |
|---------------------|--------------------------|--------------------------------|
|                     | $-\frac{dS}{dD}(\gamma)$ | $-\frac{dS}{dD}(\gamma + SEF)$ |
| 0 - 8               | $(5.0 \pm 0.5)$          | ----                           |
| 8 - 10              | $(20 \pm 1)$             | ----                           |
| 0 - 4               | ----                     | $(9 \pm 1)$                    |
| 4 - 8               | ----                     | $(14 \pm 2)$                   |

Table 1: Estimated slopes of *D. radiodurans* surviving curves  $S(D)$ , defined as their first derivatives  $dS/dD$ , for gamma irradiation only ( $\gamma$ ) and gamma irradiation plus exposure to an SEF ( $\gamma + SEF$ ).

## Discussion

### 4.1 Repairing shoulder depletion and radiobiological sensitivity

The expressive *loss of cellular viability* of *D. radiodurans* following exposure to a non-cytotoxic agent such as static electric field (SEF) combined with irradiation is highly intriguing. Interestingly, the *repairing shoulder* of the extremophilic and very high radioresistant *D. radiodurans* was reduced by a factor of 2 (from 8 kGy to 4 kGy), while for the radiosensitive *Microcystis panniformis* a reduction of only 20% was observed (from 2.5 kGy to 2.0 kGy – see figure 2 in Ref. 3). A somewhat perfunctory appraisal of their

shoulders, 8 kGy and 2.5 kGy, indicates that *D. radiodurans* is over three times more repair proficient than *M. panniformis*. However, the results show that a SEF much more intensely precludes recuperation of *D. radiodurans* after exposure to gamma radiation.

In terms of a more specific radiobiological parlance, the most relevant cell molecule is DNA. Using a simplified model for DNA damage [23], but rather useful for this discussion, the ionizing radiation was able to inflict two categories of damages. In category-I damage a single ionizing hit breaks both DNA strands (a double-strand-break, DSB) – a single event, where the average number of DSB is proportional to the dose (D). In category-II damage a single hit breaks only one of the strands (a single-strand-break, SSB), while another hit breaks the other strand (SSB) but not too far from the first – a DSB is thus produced by two independent events with a probability proportional to D.D and, therefore, the average number of DSB is also proportional to D<sup>2</sup>.

The survival fraction for the two damage categories is given by

$$S_1(D) = \exp[-\alpha D] \text{ and } S_2(D) = \exp[-\beta D^2], \quad (1)$$

with  $\alpha = 1/D_0$ , where  $D_0$  is the dose necessary to kill 63% of the sample cells in the *repairing shoulder* region.

Since the trend of S (surviving cell fraction) is driven by  $\exp[-\alpha D]$  at the lower dose irradiation regime, the *repairing shoulder* as function of D is described by a straight line in log-linear plot with a decreasing slope proportional to  $\alpha$ . In this sense, the higher  $\alpha$  is, the higher the loss of repairing capacity. A finer appraisal of the *D. radiodurans repairing shoulder* in log-linear plot after radiation and SEF exposure (see Figure 3 – insert) reveals that it is composed of two straight lines, from 0 to 4 kGy (with slope  $\alpha_1 = 0.33 \text{ kGy}^{-1}$ , that is,  $D_{01} = 3.0 \text{ kGy}$ ) and from 4 to 8 kGy ( $\alpha_2 = 1.14 \text{ kGy}^{-1}$ , that is,  $D_{02} = 0.9 \text{ kGy}$ ). When only exposed to radiation, a single straight line from 0 to 8 kGy ( $\alpha_0 = 0.24 \text{ kGy}^{-1}$ , that is,  $D_0 = 4.2 \text{ kGy}$ ) describes the entire shoulder.

The very high radio-sensitization induced by the static electric field (SEF) at doses equal and higher than 4 kGy is remarkable. In this case, a mere additional dose of 0.9 kGy kills off 63% of the cells (since  $D_{02} = 0.9 \text{ kGy}$  – see above).

#### 4.2 Radiation resistance versus DNA repair performance

There are two circumstances involved in the radioresistance performance of *D. radiodurans*.

(I) As discussed above in the *Introduction*, the wide repairing shoulder of *D. radiodurans* indicates that this

bacterium is highly repairing proficient. Such a performance is driven by protein protection against oxidative stress preserving, therefore, the efficiency of DNA repair enzymes.

(II) However, exposure to high doses of radiation (up to 8 kGy in *D. radiodurans*) leads to DNA shattering into myriads of small pieces. In this regard, it was described and demonstrated elsewhere [17] a two-stage DNA repair process for *D. radiodurans*, the ESDSA (see *Introduction*), for reassembly of hundreds of chromosomal fragments.

If (I) is accomplished but not (II) the organism is driven to death since, as pointed out elsewhere [16], a single DSB left unrepaired leads cell to death.

As observed in this work, there is a pronounced decrease in the viability of *D. radiodurans* when exposed almost simultaneously to gamma radiation and a static electric field (SEF) – see 4.1 above. Since by no means a SEF would impinge damages to proteins and/or to the DNA molecule, one is led to conclude that a SEF interferes in the DNA repairing process itself.

Altogether, the present results obtained with the radiation resistant bacterium *D. radiodurans*, plus previous results obtained at this Laboratory with prokaryotes and eukaryotes (including MRC5 lung cells) [3], [4], support the claim that post-irradiation exposure to static electric fields somehow interferes with the normal repair processes.

## Conclusions

### 5.1 Directly from experimental data

1. A Static Electric Field induces a very high radio-sensitization in *D. radiodurans*, as directly observed from the experimental results (Figure 3) and convincingly demonstrated by the statistical analysis (Figure 4).

2. A Static Electric Field negatively interferes with cell recovery processes much more intensely in the highly radioresistant *D. radiodurans* (Figure 3) than in the moderately radioresistant cyanobacterium *M. panniformis* previously studied at this Laboratory [2]. The corresponding *repairing shoulders* were reduced by 50% and 20%, respectively.

### 5.2 Inferred from data interpretation

3. The experimental results suggest that electric fields scramble reassembling of small, high-dose induced DNA fragments, therefore preventing efficacious repair processes. A model supporting such conjecture is presented in the APPENDIX.

4. The role of a Static Electric Field, evidenced by the present results, indicates that it is possible to non-invasively potentiate the effect of ionizing radiation, making any given dose more efficacious. Possible and future clinical applications would require a proper scaling investigation of the doses regime, since the high doses of ionizing radiation applied to these bacteria are not directly comparable to those in irradiation of superior eukaryotes (as e.g. mammalian cells). A lay-out of a setup, intended for possible pilot experiments and feasibility studies, is described and discussed elsewhere [27].

## Acknowledgements

This work was supported by grants from FAPESP, CNEN and CNPq, Brazilian funding agencies for the promotion of science.

## Appendix

### Proposing a Biophysical Model

The rather intriguing *massive loss of cellular viability* of *D. radiodurans* by exposure to a non-cytotoxic agent such as static electric field (SEF) combined with irradiation calls for a biophysical model explanation, as proposed below. It is important to note *ab initio* that gamma radiation shatters DNA strands [17,28] (see e.g. Figure 1-a,b of Ref. 12), while static electric fields (SEF) are merely *polarizing physical agents*, therefore playing no role in DNA damage [3,4].

Recent results on fragmentation profiles of plasmid DNA irradiated with gammas at this Laboratory, using Atomic Force Microscopy to determine fragment lengths, showed that plasmid DNA strands were highly shattered [28]. As such, genome DNA strands could be highly shattered by gammas too, as verified elsewhere with less resolution (see Figure 1 of Ref. 17). In fact, radiation senses only the density and atomic composition of the molecule [23], interacting similarly with nucleotides from either plasmid or genomic DNA. Thus, genomic DNA physical properties associated with interaction with radiation (e.g. fragmentation profiles) could be inferred from plasmid DNA.

Moreover, size distribution measurements of DNA fragments by AFM, intended for studies of DNA-radiation interaction, have been carried out only with plasmid DNA. Among the many reasons underlying this circumstance we mention the great difficulty in handling a genomic DNA for AFM imaging.

As for plasmid DNA, the molecule should be linearized to allow AFM imaging of fragment full length. Given the size of a bacterial genome, however, linearization is not feasible. Fragmentation profiles of plasmids, on the other hand, could be AFM probed with great resolution [28] *vis-à-vis* results obtained with Pulsed-Field Gel Electrophoresis [17].

Shown below (Figure A, adapted from [28]) are results for plasmid fragmentation at doses of interest for the discussion of *D. radiodurans* viability data. Scaling this scenario to the size of *D. radiodurans*, relatively smaller fragments would also be produced by gamma irradiation of the genome of this bacterium at high doses. In this sense, the sizes of these fragments would be considerably smaller than those produced in irradiations at much lower doses, as implied by the results for DNA plasmid fragment (Figure A).

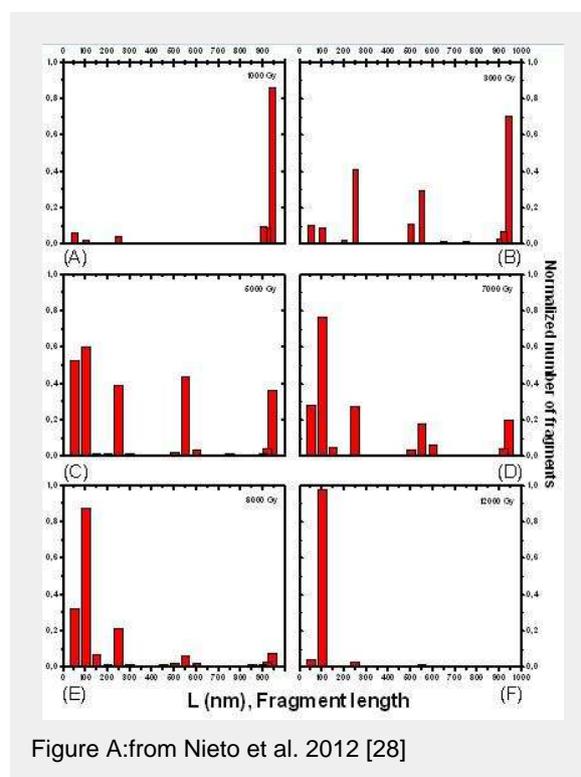


Figure A: from Nieto et al. 2012 [28]

Thus, the smaller fragments of *D. radiodurans* would much more easily be rotated and aligned with torques ( $\tau$ ) exerted by electric fields. Actually, the resulting angular acceleration ( $\alpha$ ) acquired by the fragments is proportional to  $\tau / I$ , where  $I$  is their moment of inertia. Then, smaller fragments have smaller  $I$ , and consequently larger  $\alpha$ , thus implying a more efficient alignment process. As a consequence, it would be expected that the reassembling of small fragments be far more difficult to accomplish, making deinococcal cells less able to reconstitute their shattered genomes. For the bacterium *M. panniformis*, exposed to much lower doses, the reduction of its *repairing shoulder*

was only 20%, from 2.5 to 2.0 kGy [3]. This is highly consistent with the present model, since the size of DNA fragments at lower doses is bigger (higher  $l$ ) and, therefore, much more difficult to polarize.

The survival of *D. radiodurans* relies on a repair process starting with the reassembling of these short fragments [17]. Since repair synthesis should occur before cell division, the non accomplishment of this process leads to cell lethality. In this sense, depletion of the *repairing shoulder* would mean that the capacity to rejoin shattered genome fragments has equally been depleted. Our results indicate that, on the one hand, exogenous SEF was responsible for the substantial reduction of the *D. radiodurans* repairing shoulder. On the other hand, since SEF acts only as a polarizing agent, it is very likely that DNA fragment sizes (Figure A) also play an important role, as here proposed and indicated by the results.

In fact, the shape of the curves shows (Figure 3A-insert) that the association of SEF with  $\gamma$  radiation decreases the repair capacity, as expressed by the shoulder decrease. It also shows, however, that in the low dose region (0 – 4 kGy), where the DNA strands are moderately shattered some repair does take place. Increasing the dose to a range of 4 – 8 kGy, where the DNA measured fragments are smaller (Figure A), the curve bends sharply indicating loss of repair capacity. These findings suggest that a Static Electric Field is much more effective in scrambling smaller fragments (lower  $l$ ), a circumstance rather consistent with the proposed biophysical model.

#### Checking the present biophysical model hypothesis with Static Magnetic Field

The central hypothesis of the proposed model states that SEF polarizes the displacement of fragments and other dipoles (as e.g. repair proteins) along a fixed direction which partially hampers the repair process. Static Magnetic Fields (SMF), however, allow displacements in nearly all directions since moving charges under these fields are **compelled to execute curved paths**. Unlike SEF, therefore, SMF would not appreciably interfere in the repair process.

This possibility has been verified here by means of viability measurements of *D. radiodurans* exposed to SMF after irradiation. In this regard, samples of *D. radiodurans* were exposed to a static magnetic field (SMF) of 0.4 T (4,000 G) for a period of 10 hours, inside an arrangement of two parallel layers of Neodymium-Iron-Boron magnets, following irradiations of 2, 4, 6 and 8 kGy. Results are shown in Figure B below.

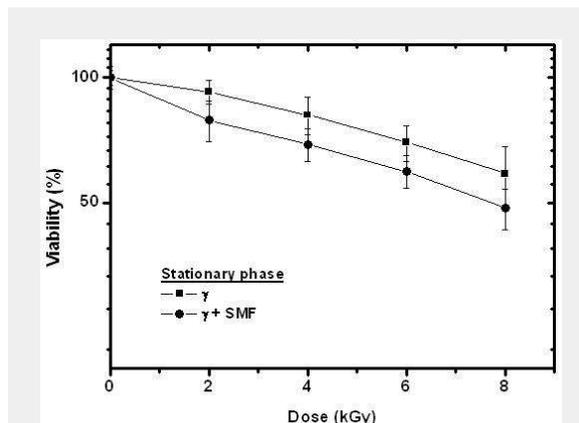


Figure B: Survival curves of *D. radiodurans* after irradiation ( $\gamma$ ) and after irradiation plus exposure to a static magnetic field (SMF) of 0.4 T by 10h. All samples were obtained during the stationary growth phase.

Observation of Figure B shows that the cytotoxicity of Magnetic Fields is very small, an indication that a SMF does not scramble DNA processes as e.g. the fragment reassembling, thus supporting the present Biophysical Model Hypothesis.

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