Classification and Comparison of Bacterial Resistance and Resistance Learning Against Low Dose Irradiation

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Abstract

Survival ability and adapting capability of microorganisms under irradiation are not fully classified so far, however, many pathways of resistance and many types of resistant bacteria have been studied previously. In this experiment, we surveyed the adaptive resistance of five Gram-negative bacteria against gamma radiation (100 Gy to 1000 Gy) which is one of the challenges facing living organisms in certain environments. Bacterial strains were standard strains taken from hospitals and research centers. Growth ability and comparison were performed by evaluating optical density measurement with a spectrophotometer. Radiation was applied up to 1000 Gy using a gamma cell in a standard irradiation facility. New findings in this experiment, besides the wide comparison of the variety of Gram-negative strains under the same irradiation process, is classifying their learning capability, which can be used to manipulate or modify some types to become resistant in a much shorter period than natural resistance learning, for special purposes. The experiment accomplished the study of their learning ability to resist the same type of ionizing radiation. Two classes were observed, those with learning ability that could become more resistant, and the other that could not learn how to resist (during the study conditions). Salmonella Typhi was the most resistant strain among the studied group (P-value < 0.005), however, unlike the Enterobacter Aerogenes and Escherichia coli, results show that S. Tiphy does not possess the adapting capability to this kind of radiation under these methodological circumstances (P-value < 0.05).

Introduction

When cells are exposed to radiation, there is an equal distribution of the radical attacks on the five carbon atoms in deoxyribose (Hagen 1994). Studies have shown damages on DNA as Double Strand Break (DSB) and Single Strand Break (SSB), and endogenously induced isolated repaired lesions very efficiently in cells by the Base Excision Repair (BER) pathway. The BER pathway is also the major pathway responsible for the repair of non-DSB clustered DNA damage sites (Lomax et al. 2013). Considering radiation's biological effects on every living cell, soil microorganisms are found to be the most resistant to gamma radiation (Denisova et al. 2007). There can also be cellular damages on bacteria caused by radiation, such as lipid peroxidation which is a highly destructive process and alters the structure and function of cellular membrane. It is involved in a number of diseases and in poisoning of several toxins (Anjali and Kale 2001). Although these radiations are malicious for bacteria, they are not fully defenseless, and resistance mechanisms are observed (Selvam 2013). Different strategies were selected during evolution including several mechanisms of radiation byproduct detoxification and subtle cellular metabolism modifications to help the cells recover from radiation-induced injuries, protection of proteins against oxidation, an efficient DNA repair tool box, an original pathway of DNA double-strand break repair, a condensed nucleoid that may prevent the dispersion of the DNA fragments and specific radiation-induced proteins involved in radio-resistance (Confalonieri and Sommer 2011). Thus, it is well understood that some bacteria can become resistant and can learn to resist gamma radiation. Regardless of what these mechanisms are, in this project, we cultured different bacteria, irradiated them at different doses, and compared their growth to achieve a comparison on their resistance against gamma radiation. We

also designed the experiment so that we can determine which one of these selected bacteria are capable of “learning” resistance against radiation regardless of how this learning is being achieved by the bacteria, i.e., where exactly the radiation is affecting and how the bacteria are repairing themselves. This resistance learning can be used for peaceful goals, for example, D. radiodurans is being engineered to express metal-detoxifying and organic compound-degrading functions in environments heavily contaminated by radiation (Brim et al 2000). Making different types of bacteria resistant to radiation also gives us a way to use them in space missions for different studies and experiments (Vaishampayan et al. 2012). It needs to be noted that by “learning to resist” we mean if they can enhance their survival at the same amount of dose, after being exposed to it previously. As an accepted concept in biology, learning can be defined as the acquisition of knowledge or skill. In this experiment, five different types of bacteria are investigated for their resistance ability to be determined and measured, and their learning capability to be indicated. Radiation resistance capabilities of Gram-positive bacteria are thoroughly known and understood, but since many types of Gram-negative bacteria possess serious health risks, it is worth studying whether they can become resistant and if it is possible to examine these bacteria in outer space. This paper is devoted to examining Gram-negative bacteria’s radiation resistance. For example, toxicity of radio-resistant Gram-negative bacteria in space stations is an important issue that needs a comprehensive survey, that is since radiation is known to change antibiotic susceptibilities of different bacteria (Kamel and Abdi 2019; Oskouee et al 2020).

Materials And Methods

This research was performed with derived bacteria samples taken from hospitals and research centers; *Salmonella Typhi* (6539) - *Escherachia Coli* (25922) - *Serratia marcescens* (13880) - *Enterobacter Aerogenes* (13048) and *Shigella flexneri* (12022), were first grown on Muller-Hinton agar plates. Then a colony was suspended in sterile Phosphate-Buffered Saline (PBS) so that its optical density absorption was between 0.08 and 0.1 read in 630 nm where the cell density (CFU/mL) could be approximately 1.5 equivalent to McFarland 0.5 Standard. This suspension was held in micro-tubes (1 ml) for irradiation. We used a cobalt gamma cell to irradiate bacteria, proposing a dose rate of 1.70 Gy/s and activity of 71 Ci. The energy range characteristic of ionizing radiation begins at about 1000 eV and reaches its upper limit at about 30 MeV (Baccaro et al. 2003; Broomfield et al. 2001). Cobalt was chosen to avoid induced radioactivity, which may appear if the gamma-ray energy is higher than 5 MeV. On the other hand, the application of lower energy radiation (below 0.2 MeV) is not rational (Aparecida and Aquino 2012). In order to make a perfect uniform allotment among micro tubes, a pillar was designed and built. The pillar was designed to arrange micro-tubes so they could be irradiated uniformly (figure 1). The final step was to compare the survival quantity after the irradiation; thus, they were planned into 96-well microtiter plates on Mueller-Hinton Agar (MHB) and PBS. Suspensions were also used to culture bacteria on MHA plates to compare the colony formations over different doses. After being held in an incubator for 8 hours, the 96-well plates were then investigated using a spectrophotometer so that the survival comparison data could be reached (McBirney et al. 2016). Results were calculated using equation 1 and can be seen in figure 2 (Armstrong and Hilton 2011). Survival rate was achieved using the following formula:

$$Survival(\%) = \frac{OD_{test}}{OD_{control}}$$ Equation 1

Where OD is the optical density read at 630 nm. In order to decrease statistical uncertainty of the measurement all experiments were repeated three times for each bacterium at each dose of irradiation. After this point of experiment, we surveyed their learning ability using adaptive response method (Streffer 2004), meaning that the irradiation processes needed to be repeated. Their last standing dose (maximum dose they could endure and survive) was chosen to take the samples from.

After a 2-month interval, these samples were adjusted in micro-tubes as before, with the same optical density absorption, and this time they were irradiated all in 300 Gy (the interval was caused by two reasons, first, the bacteria should have been left to grow and repair, and second, the radiation center schedules).

Results

A gradual decrease of survival was observed with dose increment (figure 2). All five strains (*S.Typhi, E.Coli, Serratia Marcescens, Enterobacter aerogenes, Shigella Flexneri*) showed growth ability in a 96-well microtiter after 8 hours in an incubator at 37 °C. *S.Typhi*, *Enterobacter* and *Shigella* were the most resistant bacteria against gamma irradiation. General decrease can be seen over all strain's growth as the dose increases, and colony formations are changed in different doses (figure 3).

Total loss can be seen for *E.Coli* and *Serratia* at 500 Gy, for *Shigella* and *Enterobacter* at 700 Gy and *S.Typhi* could resist gamma radiation up to more than 700 Gy (all P-values < 0.005). In order to compare their resistance learning we used adaptive response methods (repeating irradiation after a period of time) to make them resilient. After they were exposed to some point of irradiation which they could survive, they were all exposed to 300 Gy gamma radiation. They were expected to be more resistant compared to their control sets being exposed for the first time. Being able to learn, means if bacteria could

increase their survival at the same amount of dose. Figure 4 shows the survival results for the first and the second 300 Gy exposure (which was the dose that all five types could
survive considering the standard deviation).

Two types of bacteria exist in the studied group, one is capable of resistance learning (against gamma irradiation), whereas the other is not. *E.coli*, *Serratia* and *Enterobacter* belong to the first group. *Enterobacter*, for example, adapted to resist irradiation so efficiently that it had only five percent lost during the second irradiation. *S.Typhi* and *Shigella* on the other hand, showed no learning ability (P-value < 0.05) and their growth was decreased after the second irradiation. These groups are represented in the table1.

Table 1: Classification of bacteria in resistance learning ability against irradiation

Capable of resistance learning against radiation	*E.Coli*	*Serratia*	*Enterobacter*
Not capable of resistance learning against radiation	*S.Typhi*	*Shigella*	

Discussion

This paper indicates that not all types of Gram-negative bacteria are capable of adaptation or resistance learning against gamma radiation at least at the mentioned level of doses and with this method of experiment (it might be different for more cycles and/or variety doses of irradiation). As mentioned in the results, only three types of the studied group adapted themselves to resist more than the first exposure against the same amount of radiation. This experiment is suggesting that for any purpose to be accomplished using Gram-negative bacteria in a radiation exposed environment, these types of bacteria can become resistant to the proper dose of radiation in a relatively short period of time. NASA, for instance, has invested in a variety of research to evaluate the harmfulness of certain bacteria in space stations and to conduct a conclusion on their adaptability in outer space. In order to fully explore the details regarding the effects of gamma radiation on the bacteria in this experiment, different components of radiation resistance are to be surveyed separately. First, we will discuss the cell wall and outer membrane. It is mentioned in a research that Gram-positive bacteria are more resistant to UV radiation than Gram-negative ones (Romanovskaya et al. 2011). It is also discussed in a similar experiment (Williams et al. 2007) that for the germicidal radiation (UV-C) Gram-positive bacteria showed 12-13 times more resistance. This can be explained by the fact that the Gram-positive class possesses a thick cell wall (higher amount of peptidoglycan) that changes the cell susceptibilities to various environmental conditions (Mai-Prochnow et al. 2016). Similar behavior is observed in experiments regarding gamma radiation resistance (Anellis et al. 1973). This is also most importantly caused by the sulfur compounds found in the cell wall (Braun et al. 1996; Milligan et al. 1997). It is important to notice that Gram-negative bacteria, although containing a thin cell wall, still do possess this capability. Peptidoglycan recycling is also a metabolic process by which Gram-negative bacteria are able to show resistance (Mayer et al. 2019). Microscopic physical reasons for this phenomenon need further investigations concerning different physical properties of the UV light and gamma radiation interactions with bacteria. Lipopolysaccharide is another important component of the Gram-negative bacteria that is to be considered. It is known that phosphate groups of the lipopolysaccharides increase the overall negative charge, this negative charge, similar to sulfur compounds, help to stabilize the whole structure (Herrera et al. 2010) and can be consequently resistant to free radicals produced by gamma radiation. The fact that *Salmonella* is much more resistant to many antibiotics that affect cell wall synthesis (V T Nair et al. 2018; Lee 2011) (relative to other Gram-negative bacteria) indicates that its peptidoglycan recycling process and outer membrane's negative charge is relatively strong, hence, this is probably one of the reasons that *S.Typhi* is also more resistant to gamma radiation.

After covering the factors related to cell walls that were involved in radiation resistance in our results, here we discuss the genetic and DNA repair factors. It is known that oxidative DNA damage is a result of ionizing radiation. A frequent outcome of this damage is the production of 7,8-dihydro-8-oxoguanine

(GO), a mutagenic base analog (Shibutani et al. 1991), and protection against this base relies on the GO system, composed of three genes: mutM, mutT, and mutY which are present in *S.Typhi* as fpg. Oxidative damages induce a variety of defense mechanisms that in case of some mutations of *Salmonella Typhimurium* are sometimes lacking certain pathways (Garzón et al. 1996; Kokubo et al. 2005), which might be one of the most important reasons that no adapting capability was observed for this strain in this study; however, cases of radiation resistant strains of *Salmonella Typhimurium* were reported and important courses regarding their resistance were discussed (Licciardello et al 1969; Davies and Sinskey 1973). That can be explained by different environmental conditions and / or divergent genomes. There are explanations asserting the reason for *E.Coli* and *Serratia* strains being most susceptible to gamma irradiation. Since the relations between beta lactamase enzymes and radiation resistance are uncovered recently (Gaougaou et al. 2018; Yehia et al. 2020), and knowing that *E.Coli* and *Serratia* are relatively more susceptible to antibiotics linked to beta lactam mechanisms of action (Waites et al. 2006; Traub 2000) one can conclude that their weak radiation resistance is connected with this matter beside the genetic differences.

Although this strain of *E.Coli* has shown an insignificant resistance against gamma radiation, its great adapting ability can be explained by genetic adaptation related to DNA repairs [Boiteux et al. 1987; Harris et al. 2009; Byrne et al. 2014] as opposed to *Salmonella*. Also, its ability to adapt to different harsh environmental conditions such as pollution (Zhang et al. 2019), makes this bacterium another considerable candidate for radiative environment investigations next to *Enterobacter Aerogenes* that showed the highest adaptability. Other studies have indicated higher resistance of *Enterobacter Aerogenes* to Gamma radiation (Nei et al. 2012) and mentioned that hydrogen production in its cells is increased after being exposed to this type of radiation (Cheng et al. 2017) which is a considerable reason for its great resistance during the second exposure.

Declarations

Competing Interests: There is no financial or non-financial interests that are directly or indirectly related to this work.

Authors Contributions: S.O. proposed and designed the general idea of the study and wrote the main manuscript text. N.S. Designed the study and research. N.S. and S.O. performed experiments. S.A.H.F. and N.S. guided the overall activities, reviewed and edited the manuscript and provided knowledge on data interpretation as well as the advanced direction of the research. S.A.H.F. provided irradiation experiments. All authors discussed the results in detail and approved the manuscript.

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Figures

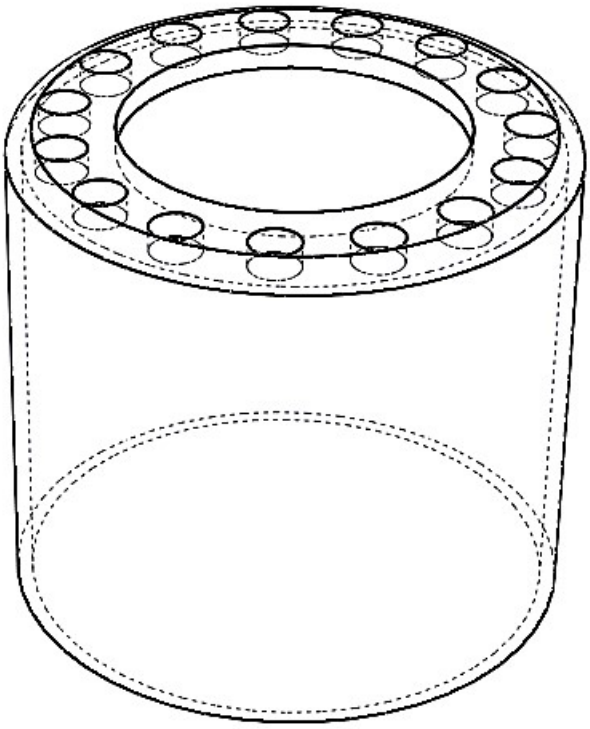

Figure 1

Designed pillar

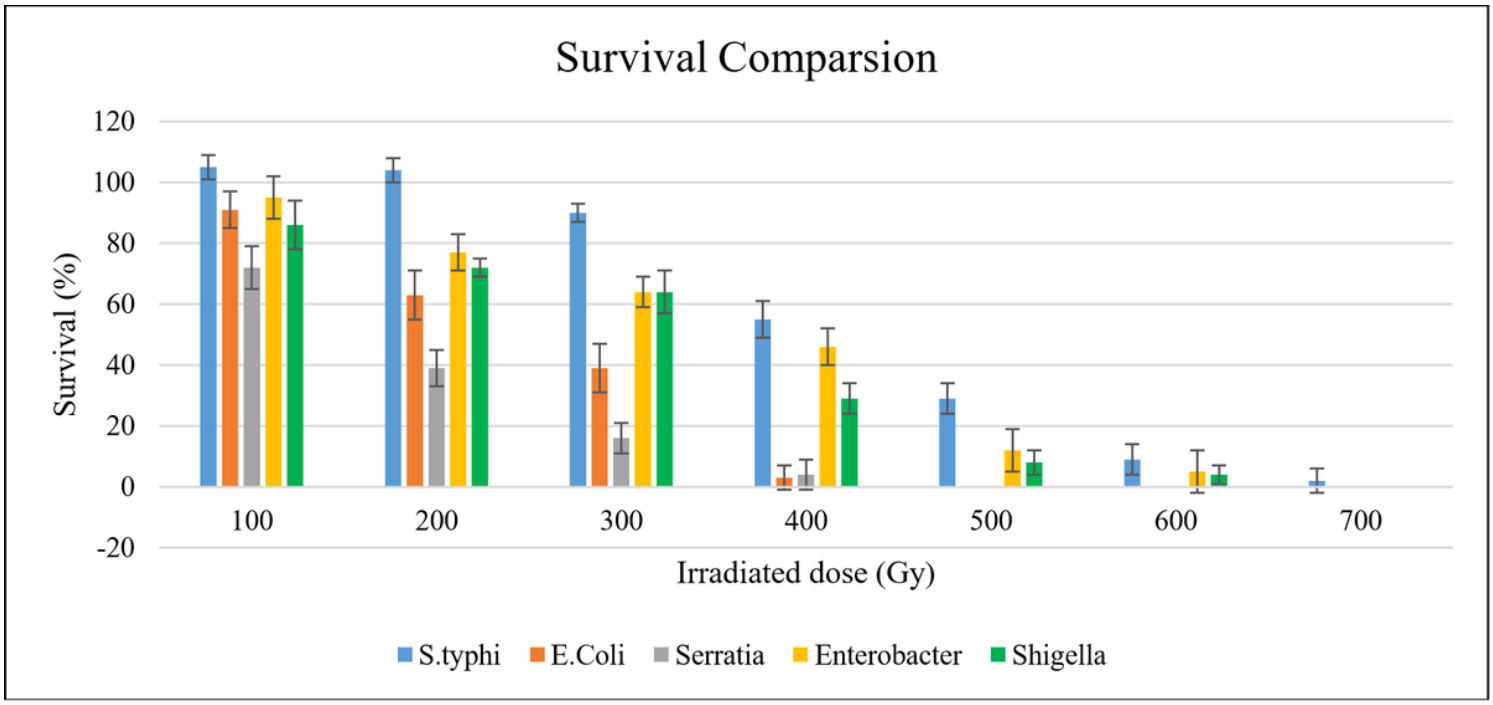

Figure 2

Survival comparison after irradiation in different doses

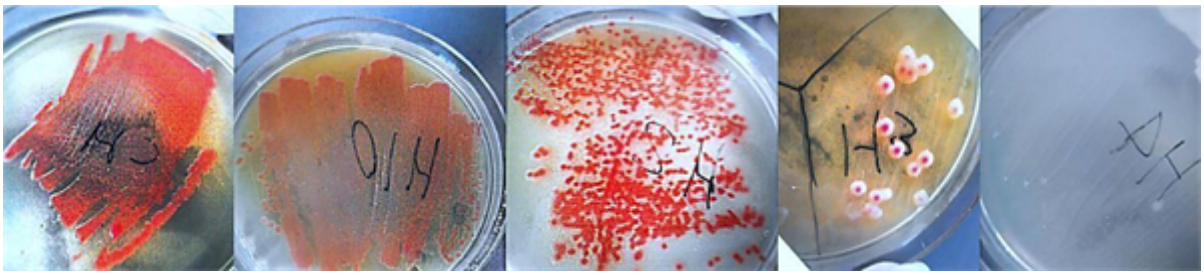

Figure 3

Colony comparison of *Serratia marcescens* in different doses of irradiation Left to right: Control set (no irradiation), 100 Gy 200 Gy, 300 Gy, 400 Gy. Changes in formation and number of colonies can be seen

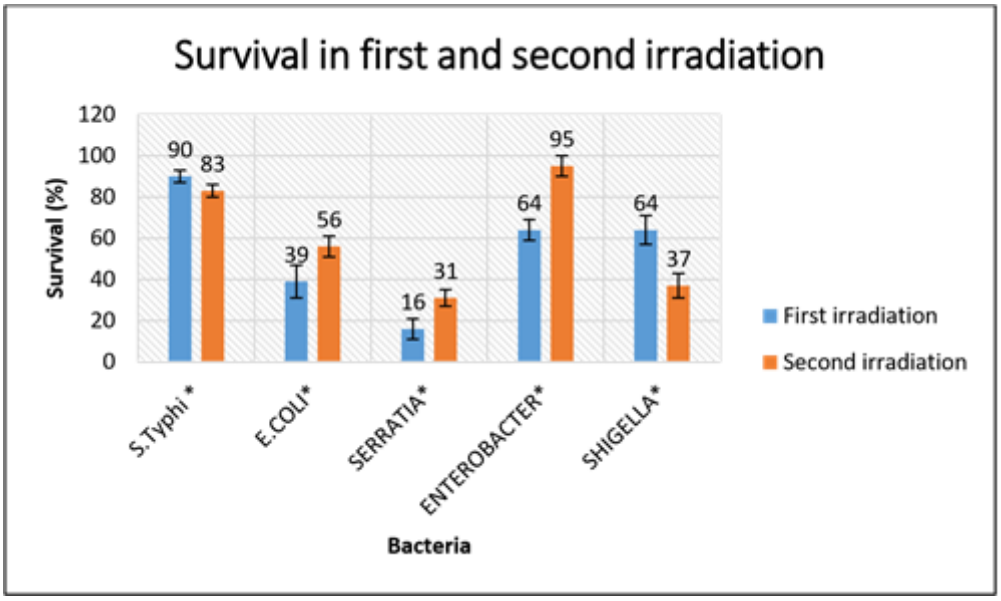

Figure 4

First and second irradiation comparison