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Research Article

Gamma Radiation Dose-Response of Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

Bacterial mutagenesis induced through gamma irradiation is one of the techniques for strain improvement. The DNA changes caused by radiation and reactive oxygen species created from water radiolysis induced bacterial mutagenesis. There is always a constant demand for better quality strains from the bioprocessing industries to speed up production and increase yield. *Bacillus* strains are Gram-positive bacteria whereas *Escherichia coli* is a Gram-negative bacteria; they are all model organisms used by the bioprocessing industries. This study investigates the effect of acute gamma irradiation on Gram-positive *Bacillus megaterium* NMBCC50018, *Bacillus subtilis* NMBCC50025 and Gram-negative *Escherichia coli*. Samples were irradiated in Gamma Cell Acute Irradiation Facility at Malaysian Nuclear Agency with irradiation doses from 0.1 kGy to 2.1 kGy. The radiation sources were from two Cesium-137 sealed sources. Dose responses are crucial information for bacterial mutagenesis studies. The survival curves of viable bacterial cell count versus radiation doses were plotted to determine dose-response and lethal dose, 50% (LD_{50}). Viable cells reduce as irradiation doses increase. The LD_{50} for *Bacillus megaterium* NMBCC50018, *Bacillus subtilis* NMBCC50025 and *Escherichia coli* were 1.2 kGy, 0.2 kGy, and 0.03 kGy, respectively. *Bacillus megaterium* NMBCC50018 was most resistant to gamma radiation. Dose responses between Gram-positive and Gram-negative bacteria were concluded to be different.

Key words: Cesium-137, ionizing radiation, mutagenesis, survival curves

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INTRODUCTION

Industries are continuously demanding better-quality microbial strains to achieve high productivity and low manufacturing cost (Leavell *et al*., 2020; Zaki *et al*., 2020). Various methods were studied to obtain improved strains for the benefit of the food, pharmaceutical, agricultural, and waste management industries (Hu *et al*., 2019; Sebastian *et al*., 2019; Venil *et al*., 2020). Besides genetic modification with biotechnology, random mutagenesis induced by ionizing radiation is a proven method to achieve strain improvement (Bouassida *et al*., 2018; Cho *et al*., 2019; Zaki *et al*., 2020). New strains obtained from classical techniques such as random mutagenesis with irradiation are regarded as non-genetically modified organisms and thus able to secure consumer acceptance and regulatory approvals (Plavec & Berlec, 2020; Hanlon & Sewalt, 2021).

Ionizing radiation excites and ionizes molecular structures by removing bound electrons causing a direct and indirect effect on bacterial DNA, leading to mutations. DNA lesions accumulated beyond the threshold level will lead to cell death. An indirect effect occurs due to the presence of free radicals (reactive oxygen species) produced by the radiolysis of water. Examples of free radicals are hydroxyl (•OH) and superoxide anion ($O_2^{-\bullet}$), which could cause oxidative stress to bacteria cells and molecules (Hashemabad *et al*., 2018; Pour Khavari, 2020). Gamma radiation, higher energy UV radiation, and X-radiation (X-ray) are the types of ionizing

radiation often used to induce mutagenesis.

Bacillus subtilis and *B. megaterium* are Gram-positive bacteria whereas *E. coli* are Gram-negative bacteria. All have been extensively studied and are often selected as the model bacterium for industry applications and research. The genus *Bacillus* is a common bacterial group in nature. *B. subtilis* is an industrial workhorse with many applications such as in enzyme production, probiotics, and food manufacturing (Gu *et al*., 2018; Errington & van der Aart, 2020) whereas *B. megaterium* is also an enzyme production strain and often selected in transformation studies (Vary *et al*., 2007). *E. coli* is the Gram-negative model used in molecular cloning and also a metabolite production strain (Chen *et al*., 2013).

The effects of radiation would be observable through molecular, biochemical, physiological, and/or morphological changes. The highly transformable *B. subtilis* type strain 168, widely used for academic and commercial purposes is the product of X-ray-induced mutations on the *B. subtilis* Marburg strain (Burkhoder & Giles Jr, 1947; Zeigler *et al*., 2008). Manikandan *et al*., (2022) subjected *Bacillus* spp. and *Streptomyces* to gamma radiation and obtained mutant strains with higher antagonistic activity towards root rot and wilt diseases. Farrag *et al*., (2019) reported changes in the outer membrane permeability of Gram-negative bacteria after gamma irradiation thus affecting antimicrobial susceptibility. Even at low doses of gamma radiation (3.8 mGy and/or 7.2 mGy), significant changes were observed in growth rate, lag phase duration, average cell surface area, cell size, and/or ATPase activity (membrane vesicles) in *B. subtilis*, *E. coli* and *Pseudomonas aeruginosa* (Soghomonyan *et al*., 2018 & Soghomonyan *et al*., 2019). Both high and low-dose radiation were used in previous studies depending on the purpose. Inactivation of bacteria requires the highest dose whereas research studies on mutagenesis applied a lower dose. Singh & Singh, (2012) sterilized bone grafts by applying 15 kGy gamma radiation for Gram-negative bacteria (such as *E. coli*) and 20 kGy for Gram-positive bacteria (such as *B. subtilis*). Manikandan *et al*., (2022) used gamma radiation 0.5 - 3 kGy for mutagenesis of *B. subtilis.*

Radioresistance of bacteria is determined by the ability to repair DNA damage and the structure of the cell wall, which functions as a protection layer against radiation (Harrell *et al*., 2018; Rohde, 2019). Gram-positive bacteria were determined to be more resistant to ionizing radiation compared to Gram-negative bacteria (Araby *et al*., 2020). Vegetative, non-spore and Gram-negative bacteria are generally more radiation sensitive compared to Gram-positive bacteria and bacteria with spores (Harrell *et al*., 2018).

Bacillus megaterium NMBCC50018 and *B. subtilis* NMBCC50025 are two Gram-positive bacteria strains recently isolated from agricultural soil with interesting properties on cellulolytic enzyme activity and bioplastic production, respectively. Gamma radiation random mutagenesis method was explored to achieve strain improvement of these high-potential strains for commercial use. Comparisons were made with Gram-negative bacteria, *E. coli* as a reference. The dose responses of Gram-positive *Bacillus* strains and Gram-negative *E. coli* towards gamma irradiation were determined in this study.

MATERIALS AND METHODS

Bacterial strains

Bacillus megaterium NMBCC50018 and *B. subtilis* NMBCC50025 were obtained from the Malaysian Nuclear Agency Bacteria Culture Collection and originated from Malaysian agricultural soil. *E. coli* DH5α was purchased from Yeastern Biotech Co., Ltd, Taiwan.

Culture conditions

Bacterial samples were cultured in nutrient broth (Oxoid, UK)/ Luria-Bertani Miller (LB) broth (Sigma-Aldrich, USA) for at least 16 h in an incubator shaker with 200 rpm at optimum growth temperature (30 °C for *Bacillus* strains, 37 °C for *E. coli*).

Growth curves

Growth curves were obtained to determine the exponential phase of the bacteria. Samples were prepared in triplicates and cell density was measured with a spectrophotometer at OD 600 nm at 0, 2, 4, 6, 8, and 24 h of incubation.

Sample preparation for irradiation

Bacterial culture (1.0 mL) at exponential phase (OD 600 nm = 0.8) was pipetted into 1.5 mL microcentrifuge tubes and centrifuged to obtain pellets. Sodium chloride 1% solution was used to resuspend bacteria cells. Samples were prepared in triplicates for each bacteria and radiation dose.

Irradiation

Irradiation was conducted in a modified Biobeam GM 8000 (Gamma-Service Medical GmbH, Germany) irradiator at the Gamma Cell Acute Irradiation Facility, Malaysian Nuclear Agency. Samples were exposed to gamma radiation emitting from two Cesium-137 sealed sources (80.7 TBq and 81.4 TBq initial activity). *Escherichia coli* was irradiated at doses of 0, 0.05, 0.10, 0.15, 0.20, and 0.25 kGy. *B. subtilis* was irradiated at 0, 0.20, 0.40, 0.60, 0.80, 1.00 and 1.20 kGy. *B. megaterium* irradiation doses were at 0, 0.30, 0.60, 0.90, 1.20,

1.50, 1.80, and 2.10 kGy. Samples were placed in random positions and arranged in beaker BB13-5 (292 mm height, 100 mm diameter) for irradiation as shown in Figure 1. The dose rate was 0.7 kGy/h. Irradiation dose started with 0 until 2.10 kGy. Samples were removed from the beaker after the specific dose was reached. The absorbed dose was determined by the Fricke dosimeter supplied, calibrated, and analyzed by Secondary Standard Dosimetry Laboratory (SSDL), Malaysian Nuclear Agency.

Fig. 1. Samples were arranged randomly in a beaker and irradiated with gamma irradiation at doses ranging from 0.1 kGy to 2.1 kGy. Fricke dosimeters were placed together with samples for dose measurements.

Survival curves and lethal dose, 50% (LD_{50})

Samples were immediately subjected to the spread plate method on nutrient agar (Oxoid, UK)/ LB agar (Sigma-Aldrich, USA) and incubated overnight. The viable cells [colony forming units (CFU)/mL] were counted. The survival curves and lethal dose, 50% (LD_{50}) were studied and compared.

RESULTS AND DISCUSSION

During each growth phase, changes in bacteria compositions and characteristics occurred. Gidden *et al*., (2009) discovered for *E. coli,* an increase in the amount of C_{cy-17} fatty acid was determined during the exponential phase whereas an increase in saturated fatty acids was detected during the stationary phase. Also, the amount of sodiated phospholipids – primarily phosphatidylethanolamines slightly decreased while the number of phosphatidylglycerols slightly increased during the stationary phase in *B. subtilis.* Bacterial compositions and specifically lipid content differences at each growth phase were expected to affect the radiosensitivity of bacteria. Sukhi *et al*., (2009) reported that *Deinococcus radiodurans* R1 was more radiosensitive at the late stationary phase. The decision was made for samples to be collected during the same growth phase, the exponential phase to ensure valid comparisons of the survival curves. Growth curves for *B. megaterium*, *B. subtilis,* and *E. coli* (Figure 2) showed similarly distinct growth phases; lag phase (0 - 2nd h), exponential phase (3rd - 8th h), and stationary phase (after 8th h). Therefore, samples were collected at OD 600 nm = 0.8 during the exponential phase (3rd - 8th h).

The number of viable cells decreased in response to increasing irradiation doses. Gamma radiation damages the DNA and molecules of cells leading to cell death. Each bacteria species and strain was affected differently when irradiated with gamma radiation. The LD_{50} for *B. megaterium* NMBCC50018, *B. subtilis* NMBCC50025, and *E. coli* were determined to be 1.2 kGy, 0.2 kGy, and 0.03 kGy, respectively in Figure 3. *B. megaterium* was the most resistant strain against gamma radiation; Gram-negative *E. coli* was the least radioresistant. Bacteria can resist radiation due to DNA repair mechanisms and protection from cell walls and composition. DNA repair mechanisms were reported to be dissimilar among each bacteria. A comparison study by Simmons *et al*., (2009) on the SOS response (bacteria response pathway to DNA damage) revealed DNA double-strand breaks caused by gamma radiation will induce a global SOS response in almost all Gram-negative *E. coli* cells but only induced SOS in a small population of the Gram-positive *B. subtilis* cells.

The major difference between Gram-positive and Gram-negative bacteria is the cell wall structure (Harrell *et al*., 2018). Gram-positive bacteria have a thicker peptidoglycan layer and strands size of 30 – 100 nm whereas Gram-negative bacteria peptidoglycan strand sizes are only around a few nanometers (Rohde, 2019). The cell wall is stabilized against radiation by sulfur compounds and the overall negative charge due to phosphate groups of the lipopolysaccharide (Oskouei *et al*., 2022). Ayari *et al*., (2009) reported an increase in fatty acids compositions and changes to the peptidoglycan of both Gram-positive *Bacillus cereus* LSPQ 2872 and Gram-negative *Salmonella* Typhi ATCC 19430 after irradiation of 1 kGy.

Bacillus megaterium was more radioresistant compared to *B. subtilis* although both are Gram-positive bacteria. Similar results were seen in a study by Honsy *et al*. (2018); *B. megaterium* was more radioresistant than *B. subtilis* on irradiated bee pollen samples. The composition of elements was suggested to be a factor causing differences in the radioresistance of bacteria. Akman *et al*. (2020) reported radiation interaction parameters of *B. megaterium* and *B. subtilis* were different due to the percentage of bacterial element compositions

such as hydrogen, carbon, nitrogen, oxygen, and sulfur. The obtained results in this study contribute to mutagenesis studies on *Bacillus* strains and *E. coli*.

CONCLUSION

It was suggested that Gram-positive and Gram-negative bacteria were affected differently when irradiated with gamma radiation. Gram-positive *B. megaterium* and *B. subtilis* were more radioresistant compared to Gram-negative *E. coli.*

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

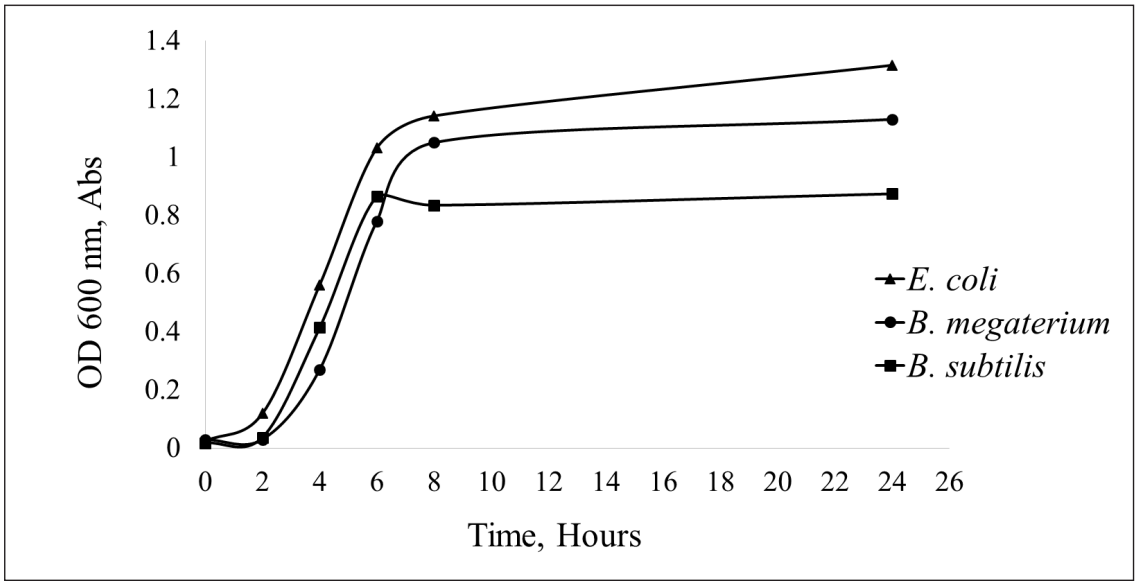

Fig. 2. Growth curves of *E.* coli, *B. megaterium* and *B. subtilis* for 24 h. The lag phase was observed at 0 - 2nd h, the exponential phase at the 3rd - 8th h, and the stationary phase after the 8th h.

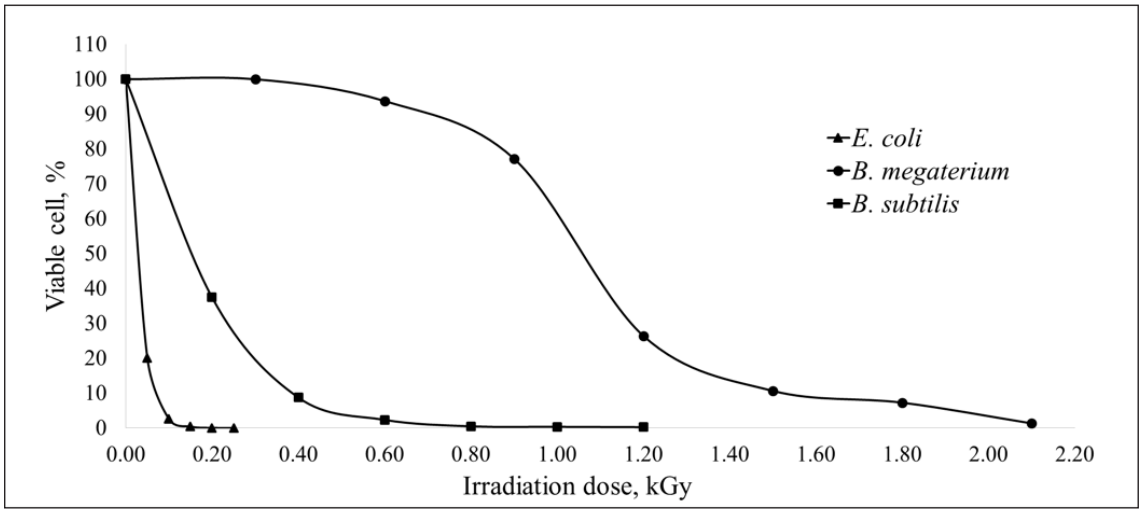

Fig. 3. Survival curves of *E.* coli, *B. megaterium,* and *B. subtilis* exposed to the increasing levels of gamma radiation from 0.1 kGy to 2.1 kGy. Surviving cells were measured as % of viable cells. The number of viable cells in samples not exposed to gamma radiation was regarded as 100%.

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