INVESTIGATION OF DNA DAMAGE AND REPAIR MECHANISM USING *DEINOCOCCUS RADIODURANS*

Lau How Mooi*, M. Kikuchi**, J. Cao***, Y. Kobayashi**, I. Narumi**, H. Watanabe**

*Special Project Division
Malaysian Institute for Nuclear Technology Research (MINT)
Bangi, 43000 Kajang, Selangor Malaysia

** Department of Biotechnology,
JAERI Takasaki, Japan

*** Medical University of Suzhou,
Suzhou, China

ABSTRACT

Deinococcus Radiodurans, formerly known as *Micrococcus Radiodurans*, is a popular bacterium because of its high resistance to damage by carcinogens such as ionizing radiation (Dean et. al. 1966; Kitayama & Matsuyama 1968) and UV radiation (Gasvon et. al. 1995; Arrage et. al. 1993). In this report, we investigated the high resistance to ionizing radiation by this bacterium. The bacteria had been exposed from 1 to 5 kGy of gamma radiation and then incubated in TGY medium to study their ability to repair the broken DNA. The repair time was measured by Pulse Field Gel Electrophoresis (PFGE) method. The repair time for each dose was determined. Also in order to ensure that the repair was perfect, the bacterium was subjected to a second exposure of ionizing radiation after it has fully repaired. It was found that the "second" repair characteristic was similar to the first repair. This confirmed that the repair after the exposure to the ionizing radiation was perfect.

INTRODUCTION

Many sicknesses, such as cancer, AIDS and some hereditary genetic disorders are attributed to the change in the DNA of the patient. Hence, great interest is now placed on investigating the mechanism of damage and repair of the DNA in the nucleus of the cell (Raymond et.al. 1995). The most common method of investigating the DNA is by studying simple DNA in bacteria to understand the basic molecular biology and chemistry. Then only it is possible to understand the more complex nature of the human DNA. In this report, the reparability of *Deinococcus Radiodurans* was investigated by Pulse Field Gel Electrophoresis method. The remarkable repair capability of these bacteria made it an interesting subject for our investigation. If the whole process of repair can be more understood from studying this bacteria, the next step would be the application of this knowledge for more practical use. This work was performed in Biotechnology Laboratory of Japan Atomic Energy Research Institute (JAERI), Takasaki, Japan. Another group of scientists in this laboratory was trying to isolate the genes

responsible for this remarkable repair and transfer it in normal E-coli. Preliminary results showed that the resultant E-coli also became radiation resistant (Barrows 1989). Investigation of this nature was still in progress and more results would be forth coming from this laboratory.

MATERIALS AND METHODS

1.0 ml of frozen glycerol stock *D. Radiodurans* MR1 cell was inoculated into 150 ml of TGY liquid culture medium (0.5% bacto-tryptone, 0.3% bacto-yeast extract, 0.1% glucose in distilled water and at pH 7.0). The bacteria were allowed to grow for about 14 hours at 30 °C with strong shaking for good aeration. The bacteria were harvested at the exponential growth phase for this experiment. The volume of the culture solution to be used as one sample was determined by measuring the light transmission using a spectrophotometer, as 710 µl solution with Optical Density of 1.0 by 660 nm light is equivalent to 4×10^9 bacteria cell. This volume of TGY was filtered through Millipore filter paper of pore size 0.22 µm and diameter 25 mm using specially made suction apparatus. The cells were washed twice with 10 mM phosphate buffer (PB). The total cells of about 4×10^9 were used as one sample for irradiation. The bacteria on the filter paper were placed on PB agar gel (2%) for irradiation.

After the irradiation, the cells were transferred to TGY agar gel (2%) plates for the bacteria to repair the damage in the incubation chamber at 30 °C. Timed samples were taken from the TGY agar gel plates in order to determine the time required for the bacteria to fully repair itself.

These samples were than washed with PB buffer and finally with Multibuffer to be made into agarose plugs for enzymatic action, such as the lysozyme to destroy the cell wall and proteinase-K to destroy the proteins. Then the plug was treated with *Not* 1 restriction endonuclease to cut the genomic DNA into the banding pattern to be measured using Pulse Field Gel Electrophoresis (PFGE) by Geneline™, Beckman (Contopoulou et. al. 1987). The plugs were cut so that it can be fitted into the agarose gel mold and electrophoresesed for 16 hours at 8 °C of TAFE electrolyte. The DNA in the gel can be stained by Ethydium Bromide (EtBr) and photographs of the DNA banding pattern can be made as shown in Figure 1.

RESULTS AND DISCUSSION

Figure 1 showed the results of the stages of repair for irradiation with gamma ray of 1.0 kGy, with respect to time of incubation in TGY medium at 30 °C, this is also the time after the irradiation, for the *D. Radiodurans* to repair itself. From this figure, it can be concluded that after 100 minutes, the *D. Radiodurans* was fully repaired by comparing with the unirradiated sample as the control. Immediately after the irradiation, the bacteria's DNA was badly broken up as can be seen in the lane with 0.0 minutes and compare with the unirradiated sample as control. Slowly, the brightness at the bottom of the subsequent lanes diminished because more and more of the DNA's are partially

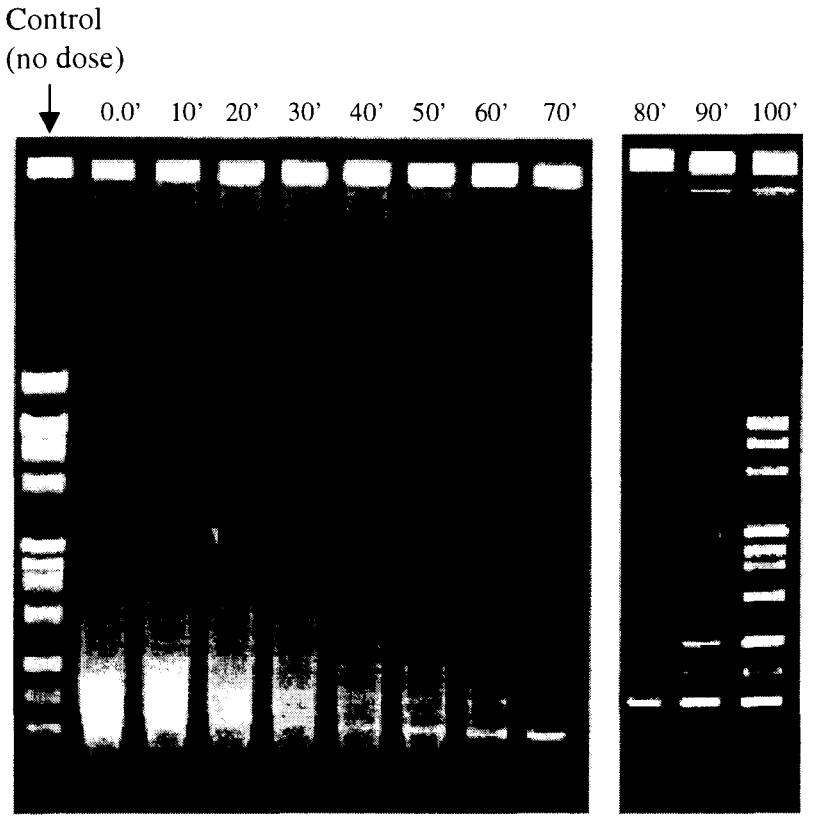

Figure 1 The repair time in minutes for *D. Radiodurans* in TGY after 1 kGy of Gamma Irradiation

repaired. In the lane for 70 minutes, the smallest DNA band at the bottom showed very distinctly. Then as the time progressed, more and more bands started to appear, and at 100 minutes, the bacteria was fully repaired because all the banding pattern appeared same as the controls. Table 1 showed the summary of the results of the irradiation dose and the repair time by *D. Radiodurans* to Co-60 gamma radiation.

Table 1 The repair time in hours after the *D. radiodurans* was exposed to different doses of gamma radiation.

Dose, kGy	1.0	2.0	3.0	4.0	5.0
Repair time, Hr	1.7	3.0	3.5	4.5	5.2

In order to be sure that the DNA repair was perfect, the bacteria was subjected to a second irradiation after the full repair to study the 'second' repair characteristic. Figure 1.2 showed that the second repair was almost similar to the first repair, as the 100-minute lane showed that the bacteria had fully repaired itself. This proved that the bacteria has fully recovered after the first irradiation and showed the second irradiation effects were the same as the first.Table 2 showed the summary of the results of the irradiation dose and the second repair time by *D. Radiodurans* to Co-60 gamma radiation.

Table 2 The second repair time in hours after the *D. radiodurans* was exposed to different doses of gamma radiation

Dose, kGy (First; Second)	1.0; 1.0	2.0; 2.0
Second Repair Time, Hr.	1.7	3.0

CONCLUSION

This experiment showed that if ample time was given to *D. Radiodurans* to repair itself after subjecting the bacteria to high doses of ionizing radiation, the bacteria can repair itself. The second part of the experiment confirmed that the repair was perfect because the second repair characteristics were the same as the first.

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