Effects of γ ray irradiation on Vibrio Qinghaiensis sp. Q67

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Abstract. In order to investigate the luminous responses of γ ray irradiation on Vibrio Qinghaiensis sp. Q67, two γ ray sources, 60Co and 137Cs, were used. Following the dose rates between 0.05Gy/min and 0.2Gy/min of 60Co, the relative luminous value (RLV) of Q67 was less than 1 after 5 minutes irradiation and inversely related to dose rate. Irradiated 1 hour at dose rates range from 100nGy/h to 10mGy/h of 137Cs, two successive stages in the luminous response were found: hormesis and inhibition. It was found that RLV was interleaved and could not be distinguished until inhibition stage appearance. The time when RLV drops to less than 1 (T0) was linear with the logarithm of dose rate. Experimental result indicates that Q67 is sensitive to acute γray radiation, which could be used to monitor γray radiation.

1. Introduction
Increase of the application of nuclear energy makes the effects of ionizing radiation on living organisms very important. Scientifically monitoring and evaluating the radiation hazard is a challenge for researchers working in related field. Traditional methods monitored by a radiation monitor or an ion concentration are accurate enough but difficult to judge the biological effects of radiation, which makes bio-toxicity detection method favored. Luminescent bacteria test, for its simplicity, rapidity, sensitivity and availability has been used as a bioassay to investigate the toxicity of heavy metal, pesticide, antibiotics for almost half of a century [2-4]. However, biotoxicity of ionizing radiation is still being studied, and the research objects are marine luminescent bacteria: Vibrio fischeri and Photobacterium phosphoreum T3, spp. [5-8], rarely on the freshwater luminescence bacteria: Vibrio Qinghaiensis sp. Q67.

Thus, the purpose of this study is to investigate the response of Q67 to γ radiation, and to provide some up-front data for monitor radioactive toxicity.

2. Materials and Methods

2.1. Bacteria
Vibrio Qinghaiensis sp. Q67, freeze-dried as pellets in glass bottles, was purchased from Beijing Hammatsu Photon Techniques Inc., Beijing, China.

2.2. Instruments
Luminometer (BHP9514), provided by School of Ecology and Environmental Sciences, East China Normal University. 60Co γ ray source(5 x 10^13 Bq), 137Cs γ ray source (3.7 x 10^11 Bq), provided by Shanghai Institute of Measurement and Testing Technology.

2.3. Toxicity tests
For the bioassay test, the Q67 freeze-dried as pellets in glass bottles were removed from -20°C storage before the test, with recovery liquid (0.8% sodium chloride solution) added for rehydration at 20°C for 15 min. The standard methods for culture medium preparation and Q67 incubation were adopted from a previous article [2].

The assay of the Q67 was carried out by 25ul bacterial suspension and 2ml 0.8% sodium chloride solution to a test tube. For each test, six test tubes were prepared, three for parallel samples and three for blank control. Parallel samples were irradiated in room air and subsequently kept at 10℃, control tubes in the same conditions but absence of irradiation.

In the toxicity tests, eight dose rate gradients were designed. The parameters of each test were shown in Table 1.

| Test No. |  γ  ray source | Dose rate | Irradiation time |
|---------|---------------|-----------|-----------------|
| 1       |  60Co        | 0.05Gy/min | 5min            |
| 2       |  60Co        | 0.1Gy/min  | 5min            |
| 3       |  60Co        | 0.2Gy/min  | 5min            |
| 4       |  137Cs       | 100uGy/h   | 1h              |
| 5       |  137Cs       | 500uGy/h   | 1h              |
| 6       |  137Cs       | 1mGy/h     | 1h              |
| 7       |  137Cs       | 5mGy/h     | 1h              |
| 8       |  137Cs       | 10mGy/h    | 1h              |

Results were evaluated by relative luminous value ($R_{LV}$), which was calculated as

$$R_{LV} = \frac{I_{irra,t}}{I_{0,t}}$$

where $I_{irra,t}$ is the bioluminescence intensity at t minutes after irradiation of Q67 in the test sample; $I_{0,t}$ is the bioluminescence intensity at the same time of Q67 in the control sample [1].

Each test was repeated thrice, and the average $R_{LV}$ was taken as the final result.

3. Materials and Methods

3.1. Effects of high dose rates γ irradiation of $^{60}$Co source

Q67 irradiated 5 minutes by $^{60}$Co at 0.05Gy/min, 0.1Gy/min and 0.2Gy/min dose rates. Figure 1 illustrated the relative luminous value ($R_{LV}$) after irradiation. Seen from figure 1, $R_{LV}$ being less than 1 suggested that those dose rate γ irradiation induced acute toxicity to Q67. $R_{LV}$ continued decline as the time after irradiation went on. However, the decline rate slowed down.

It also can be found that the higher the dose rate, the smaller $R_{LV}$. Selected the relative luminous value at 15 minutes after irradiation ($R_{LV,15}$) as the study target. The curves of $R_{LV,15}$ over dose rate as shown in Figure 2.
The greater dose rate, which means that more γ photons impinge the Q67 suspension per unit time, makes more free radicals and peroxides are produced in indirect inactions of radiation, and increasing probability of direct radiation damage to protease and DNA during a short time [12]. Therefore, the relative luminous value declined rapidly with the increase of dose rate.

3.2. Effects of low dose rates γ irradiation of 60Cs source

Irradiated 1 hour with low dose rates of 137Cs, the curves of relative luminous value (RLV) with the time after irradiation were present in Figure 3. From the graph, RLV was greater than 1 in the initial period after irradiation at the dose rates range of 100μGy/h to 10mGy/h. Then RLV declined as an overall trend whereas fluctuated locally, and RLV at different dose rates could not being distinguished until it dropped down to 1.

For conveniently describing RLV variation, two main stages were divided: hormesis stage (RLV >1) and inhibition stage (RLV <1).

Among hormesis stage, three stages can be subdivided: initial stage, recovery stage and decline stage. Q67 stimulated by γ rays during irradiation which leaded RLV reaching a certain point at the end of irradiation. In initial stage, RLV decline resulted from stimulus disappearance. The period of RLV fluctuated was described as recovery stage. In decline stage, RLV fell again, but still greater than 1.

Inhibition stage occurred when the RLV was less than 1 as RLV persistently decreased. It can be found that RLV<1 appeared earlier at higher dose rate. The specific division of each stage was listed in Table 2.

Define the time when relative luminous value dropped to 1 as transition time (T0). The values of T0 in Table 2 showed that T0 decreased with the increase of dose rate, and met logarithmic relationship (Figure 4).

Seen from Figure 3, after the T0 in inhibition stage, the decline rate of relative luminous value increased as the dose rate increased. Fitting with SPSS19.0, the liner relationship between relative luminous value and time in the period after T0 was found. Fitting functions were seen in Table 2.
The reason for luminous intensity enhanced ($RLV > 1$) of Q67 at low dose rates $\gamma$ irradiation between $100$uGy/h and $10$mGy/h may be radiogenic metabolism [13]. Q67 transforms radiant energy into chemical energy, which promotes the metabolism of life and stimulates the fluorescent enzymes in bacteria. However, stimulation accelerating consumption and lack of culture medium made relative luminous value reduced.

4. Conclusion

In this paper, the effects of $\gamma$ radiation dose rate on the luminous intensity of Q67 were investigated by using the two kinds of $\gamma$ ray sources: $^{60}$Co and $^{137}$Cs. $^{60}$Co $\gamma$ ray source provided high dose rate ($0.05$Gy/min - $0.2$Gy/min), Q67 irradiated 5 minutes, luminous intensity was restrained immediately. In the 15 minutes after irradiation, the relative luminous value ($RLV_{15}$) of Q67 was linear with the logarithm of dose rate ($d$) (Seen in Figure 2).

The low dose rates ($100$uGy/h - $10$mGy/h) irradiation, provided by $^{137}$Cs, initiated two stages in Q67 bioluminescent kinetics: hormesis and inhibition (Figure 3 and Table 2). In hormesis stage, the relative luminous value of Q67 was interleaved and could not be distinguished. However, as the time after irradiation went on, differences were gradually reflected in inhibition stage. It also found that the time when $RLV$ drops to less than 1 (transition time ($T_0$)), was linear with the logarithm of dose rate (Figure 4).

**Figure 3.** Effects on relative luminous value ($RLV$) of Q67 after irradiation by $^{137}$Cs

**Figure 4.** Relation between transition time ($T_0$) vs. dose rate ($d$)

**Table 2.** Division of stages in the luminous response after irradiation

| Dose rate | Hormesis stage | Inhibition stage |
|-----------|----------------|------------------|
|           | Initial stage  | Recovery stage   | Decline stage | $T_0$ | Fitting function $RLV$ vs. $t$ |
| 100uGy/h  | 5-10*          | 10-55            | 55-120        | 125*  | $RLV = -0.0012t + 1.147$ |
|           |                |                  |               |       | $R^2=0.99$                   |
| 500uGy/h  | --             | 5-45             | 45-105        | 110   | $RLV = -0.0015t + 1.162$ |
|           |                |                  |               |       | $R^2=0.98$                   |
| 1mGy/h    | 5-10           | 10-30            | 30-90         | 100   | $RLV = -0.0022t + 1.207$ |
|           |                |                  |               |       | $R^2=0.96$                   |
| 5mGy/h    | 5-30           | 30-40            | 40-85         | 90    | $RLV = -0.0024t + 1.184$ |
|           |                |                  |               |       | $R^2=0.97$                   |
| 10mGy/h   | 5-75           | --               | --            | 80    | $RLV = -0.0029t + 1.180$ |
|           |                |                  |               |       | $R^2=0.98$                   |

* The beginning and ending time of each stage after irradiation (unit: min).
** The time required for $T_0$ (unit: min).
The experimental results showed that Q67 was sensitive to γ irradiation. When the dose rate was high, the luminous intensity of Q67 was suppressed for a short time irradiation, and the relative luminous value of 15 minutes after irradiation could be used as the index value to monitor γ ray toxicity. For low dose rates γ ray, Q67 should be irradiated longer time, and for longer time to measure the luminous intensity after irradiation until relative luminous value fall below 1. $T_0$ was the index.

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