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Bacterial sensitivity to UV light as a model for ionizing radiation resistance

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Summary

Six bacterial isolates from the U.S. DOE Subsurface Science Program and three reference bacteria were tested for resistance to UV light and gamma radiation. The subsurface isolates included three aerobic, pigmented, Gram positive bacteria and three microaerophilic, non-pigmented, Gram negative bacteria. *Deinococcus radiodurans* was the most resistant bacterium to both types of radiation, with a D_{37} value of 4.0×10^4 μWs cm^{-2} to UV light and 300 krads to gamma radiation. The aerobic subsurface bacteria were found to be significantly more resistant ($p < 0.05$) than the microaerophilic subsurface bacteria to UV light and gamma radiation. Due to the similarities of bacterial survival between UV and gamma radiation; it is proposed that UV light could be utilized to model the fate of microorganisms exposed to ionizing radiation.

Key words: Aerobic bacteria; Microaerophilic bacteria; Gamma radiation; Ionizing radiation; Subsurface; UV light

Introduction

The presence of mixed organic and radioactive wastes in subsurface sediments and groundwater aquifers have prompted studies into the geology, hydrology and microbial ecology of subsurface environments [1]. Recent investigations sponsored through the U.S. DOE Subsurface Science Program, have revealed metabolically active, heterogenous microbial communities widely distributed throughout sediment strata to depths exceeding 500 m [2–6]. These include aerobic [7] and microaerophilic [8] chemoheterotrophic bacteria as well as anaerobic methanogens and sulfate-reducing bacteria [9]. Microorganisms in the subsurface may have a potential role

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in the in situ bioremediation of mixed wastes containing organic and radioactive components. For example, Lovley and Phillips [10] have shown that the sulfate-reducing bacterium *Desulfovibrio desulfuricans* is capable of precipitating uranium(IV) through the reduction of soluble uranium (VI).

Subsurface bacteria possess a diverse range of metabolic activities [9,11]; but little is known about the survival characteristics of subsurface microorganisms. Knowledge about survival in radioactive environments would be useful to evaluate the success of in situ bioremediation in these areas. An important component of a cell's defense against toxic chemical and radioactive agents is the ability to repair damage to its genetic material. DNA repair processes may be critical in environments containing mixtures of organic and radioactive wastes since DNA damage results from radiation generated by decaying radionuclides [12,13]. Organisms under consideration for bioremediating mixed wastes should have the capacity to tolerate and/or repair DNA lesions that would otherwise lead to mutation or death.

Two obstacles limiting the testing of bacterial responses to ionizing radiation are cost and source access. A potential solution is the utilization of cheap, readily available DNA-damaging agents such as UV light to model the effects of ionizing radiation on microbial survival. UV light in the 200–300 nm range inflicts damage to bacterial DNA and RNA [13,14]. Many of the molecular mechanisms that repair UV damage are identical to or overlap with those involved in the repair of ionizing radiation damage [12]. Therefore, in some instances, UV light may be useful in modeling the capacity of a bacterium to tolerate ionizing radiation.

In this study, six bacterial isolates from the DOE Subsurface Microbial Culture Collection (three aerobic gram positive strains and three microaerophilic gram negative strains) were exposed to UV light and gamma radiation. Bacterial survival curves were compared to determine the validity of substituting less expensive protocols such as UV exposure for modeling ionizing radiation resistance. Subsurface bacterial survival to radiation was also compared to the survival of three reference bacteria: *Escherichia coli* ATCC 25922, *E. coli* B and *Deinococcus radiodurans* ATCC 13939.

Materials and Methods

Bacterial isolation procedures

Subsurface soil samples were obtained through the Subsurface Science Program of the U.S. Department of Energy at the Savannah River Laboratories. A thorough description of the drilling sites and protocols have been detailed elsewhere [15]. Soil samples from depths of 150–500 m served as the source for primary isolation of bacterial colonies. The isolation medium was Dilute-Substrate Mineral Salts (DSMS) [8]. Pure colonies were isolated from soil-inoculated MPN tubes which contained either DSMS broth or DSMS semi-solid media (DSMS + 1.5 g l^{-1}. Agar Noble [Difco]), or from agar plates (DSMS broth + 15 g l^{-1} Agar Noble). Subsurface strains were identified as being microaerophilic based on characteristic banding properties when inoculated into DSMS semi-solid agar tubes. These isolates also exhibited spreading motility on aerobically incubated DSMS agar plates [8]. All subsurface isolates were maintained aerobically on DSMS plates at 25°C.

The reference bacterial strains used as controls in this study included *Deinococcus radiodurans* ATCC 13939, *Escherichia coli* ATCC 25922 and *E. coli* B (obtained from the VPI & SU culture collection). All reference bacteria were maintained at 30°C on T-soy agar (Difco) plates.

UV apparatus

Samples were UV irradiated in a 46 × 15 × 30 cm foil-lined plexiglass box which contained a 15 W, 254 nm UV light source (NIS germicidal lamp) affixed at the top. The light source was positioned 34 cm from the samples. The UV fluence rate (energy per area per time) incident to the test sample was measured with a UVX Radiometer (UVP Inc., San Gabriel, CA) in units of $\mu W\ cm^{-2}$. The fluence rate was modified by positioning the samples along the length of the box. There was no detectable deviation in the fluence rate over time. Total UV dose was determined by time of exposure to the UV fluence rate in units of $\mu Ws\ cm^{-2}$. All UV irradiation procedures were performed under red light to prevent photoreactivation.

UV irradiation of cultures in phosphate buffer

Stationary phase bacterial isolates suspended in 0.02 M phosphate buffer (pH 7.0) were used in UV irradiation tests. Subsurface cultures grown in DSMS broth (which was modified with 0.25 $g\ l^{-1}$ each of glucose, peptone, tryptone and yeast extract) and reference bacteria grown in T-soy broth, were centrifuged for 15 min at 5000 × *g*, washed twice and suspended in buffer to a final concentration of 10^6–10^8 cells ml^{-1}. Aliquots (2 ml) of each suspension were transferred to 60 mm petri plates resulting in a depth of less than 3 mm of liquid. The open petri plates were exposed to UV fluence rates of 300–700 $\mu W\ cm^{-2}$ with the total dose being a function of fluence rate and time of exposure. Each bacterial suspension was irradiated individually. Cultures were manually agitated during UV exposure to prevent the settling of cells.

Following irradiation, a 1.0 ml aliquot of each suspension was serially diluted in 0.02 M phosphate buffer and plated. The plating medium for subsurface isolates was modified DSMS agar and the reference bacteria were spread-plated onto T-soy agar. The plates were wrapped in foil and incubated for up to ten days. The incubation temperature was 25°C for soil isolates and 30°C for reference bacteria. Percent survival at each dose was determined by comparing colony counts of irradiated cells to a non-irradiated control.

Gamma irradiation

A ^{60}Co ionizing radiation source located at Oak Ridge National Laboratory (ORNL) (Oak Ridge, TN) was used to test the survival of subsurface bacteria to ionizing radiation. The activity of the source was 4.1 kCi with a dose rate of 350 krads h^{-1}.

Log-phase bacterial cultures were centrifuged for 30 min at 3000 × *g*, washed twice with and suspended in 0.02 M phosphate buffer. Two ml aliquots of the suspensions were transferred to 15 ml screw-capped tubes and placed on ice in the dark for transportation to the radiation source.

The cultures were irradiated in groups of 7 or 8 closed screw-capped tubes for 0–120 min. Immediately following irradiation, the tubes were placed on ice and in the

dark until the diluting and plating procedures could be accomplished. The average time between irradiation and plating was 2 h. The incubation times and temperatures were identical to those described for UV irradiation. Percent survival was determined at each exposure time.

Calculations

To compare the resistance levels of the bacterial strains to radiation treatments, D_{37} and D_{10} values were calculated. Sensitivity to radiation is often described by the D_{37} value [16,17] which is defined as the radiation dose required to inactivate 63% of a bacterial population, or that required to kill one viable unit. The D_{10} value is the radiation dose which inactivates 90% of the bacterial population.

D-values from both shouldered and non-shouldered survival curves were calculated as described by Harm [16]. The equation used for a non-shouldered survival curve was:

$$S/S_0 = e^{-cF} \qquad (1)$$

where S/S_0 was the percent survival at dose F; and $-c$ was the slope of the exponential portion of the curve. Therefore, the *D*-value is equivalent to F if the percent of survival and the slope of the survival curve are known.

The equation used for a shouldered survival curve was:

$$n = e^{F_T/F_{0.37}} \qquad (2)$$

where n was the ordinate axis intercept of the regression line for the exponential portion of the curve; F_T was the threshold dose (a measure of the length of the shoulder); and $F_{0.37}$ was the dose calculated in equation [1] which resulted in a 37% survival rate of the population. The *D*-value, in the case of a shouldered survival curve was defined as the sum of F (from equation [1] and F_T (17).

Results and Discussion

UV irradiation of bacteria

The most UV resistant bacterium in this study was *D. radiodurans*. This bacterium is Gram positive, red-pigmented and exceptionally efficient at repairing radiation-induced DNA lesions [17,18]. The survival curve for *D. radiodurans* (Fig. 1) exhibited a very gradual slope of -0.03 (Table 1) within the range of UV doses tested. This resulted in D_{37} and D_{10} values of 4.0×10^4 μWs cm^{-2} and 9.1×10^4 μWs cm^{-2} respectively (Table 2). The D_{37} value for *D. radiodurans* was similar to those previously reported which range from $3.5–6.0 \times 10^4$ μWs cm^{-2} [17].

Unlike *D. radiodurans*, the Gram positive, aerobic, subsurface bacterial isolates, UV1, UV2 and UV3 demonstrated exponential killing kinetics once a threshold UV fluence was reached (Fig. 1). Survival curves from all three aerobic subsurface bacteria were similar in that they were composed of two distinct components. There was an area of near zero slope at low UV doses termed a shoulder, and a linear portion of the curve where an exponential rate of killing was achieved. Damage

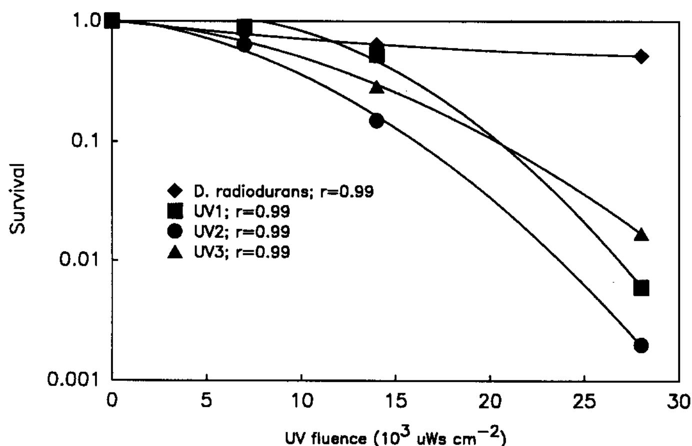

Fig. 1. Survival curves of *D. radiodurans* and three aerobic subsurface bacteria designated UV1, UV2 and UV3 exposed to 254 nm UV light. Total dose was a function of the fluence rate (700 μW cm^{-2}) and time of exposure.

inflicted by radiation doses in the shoulder region of the survival curve is thought to be enzymatically repaired, resulting in a decreased level of cellular lethality. However, as the radiation dose is increased, DNA lesions accumulate until the cell's repair functions and other protective mechanisms are overwhelmed [13]. Past the threshold of UV tolerance, further increases in UV dose result in an exponential loss of viability.

The threshold dose (F_T) for the aerobic Gram positive subsurface isolates ranged from 4000 to 8000 μWs cm^{-2}. The UV resistance of the aerobic subsurface bacteria was significantly less than that of *D. radiodurans* which exhibited a relatively low rate of killing. For *E. coli* B, the UV survival curve had an exponential, linear killing rate (Fig. 2) with a slope of −0.59 (Table 1). The D_{37} of *E. coli* B was calculated to be 1.7

TABLE 1

Slopes[a] of bacterial survival curves to UV and gamma radiation

Organism	UV	Gamma
D. radiodurans	−0.03 ± 0.01	−0.004 ± 0.001
E. coli B	−0.59 ± 0.06	ND[b]
E. coli ATCC 25922	ND	−0.55
UV1	−0.28 ± 0.07	−0.24
UV2	−0.30 ± 0.11	−0.09
UV3	−0.26 ± 0.11	−0.09
M1	−0.52 ± 0.04	−0.42
M2	−0.54 ± 0.04	−0.46
M3	−1.2 ± 0.29	−0.43

[a]Slope calculated from the exponential portion of the survival curve.
[b]Not determined.

TABLE 2

D-values[a] (10^3 μWs cm^{-2}) from bacterial survival curves to UV and gamma radiation

Organism	UV		Gamma	
	D_{37}	D_{10}	D_{37}	D_{10}
D. radiodurans	40 ± 13	91 ± 29	300 ± 53	640 ± 180
E. coli B	1.7 ± 1.4	4.0 ± 0.35	ND[b]	ND
E. coli ATCC 25922	ND	ND	3.6	8.2
UV1	12 ± 0.82	17 ± 1.7	12	17
UV2	8.4 ± 2.6	13 ± 1.7	11	26
UV3	10 ± 4.5	17 ± 7.3	11	26
M1	1.9 ± 0.15	4.5 ± 0.38	2.4	5.5
M2	1.9 ± 0.14	4.3 ± 0.28	2.2	5.0
M3	1.3 ± 0.14	2.4 ± 0.49	2.3	5.3

[a]*D*-values were defined as the UV fluence which reduced a cell population to a specified percentage of the original number of cells. The *D*-values were calculated from the regression line of the exponential slope of the survival curve as described in Materials and Methods.
[b]Not determined.

× 10^3 μWs cm^{-2}; which indicated this bacterium was more than 20 × more sensitive to UV than *D. radiodurans*. *E. coli* B was not able to significantly repair damage even at the lower UV doses tested (no observed shoulder). However, the lack of a shoulder does not necessarily signify an absence of repair activity. *E. coli* B is considered repair competent, and a shouldered region would probably have been observed if lower UV dose levels had been used [19].

UV survival curves for the microaerophilic subsurface bacteria were similar to those for *E. coli* B; demonstrating a linear decrease in viability as the dose was increased, with no evidence of a shoulder region (Fig. 2). These bacteria exhibited survival curves with slopes approximately twice as steep as the aerobic strains (Table 1). The microaerophiles were significantly more sensitive to UV light than the aerobic isolates as determined by a Student's *t*-test ($p < 0.05$) of the *D*-values calculated from the bacterial UV survival curves (Table 2). While the aerobic subsurface isolates exhibited D_{37} values to UV light between 8000 and 12 000 μWs cm^{-2} (Table 2), the microaerophilic isolates had D_{37} values of approximately 2000 μWs cm^{-2} or less. These observations indicated the greater capacity of the Gram positive aerobic strains to resist the DNA damage inflicted by UV light.

Gamma irradiation of bacteria

The results of bacterial exposure to gamma radiation were similar to those obtained with UV light (Fig. 3–5) in that *D. radiodurans* was the most resistant bacterium with a slope of −0.004 (Table 1), which was an order of magnitude more resistant than those of the subsurface bacteria. The large shoulder region in the *D. radiodurans* survival curve was most likely the result of the excision repair mechanism, which has been shown to efficiently repair radiation-induced lesions in this bacterium

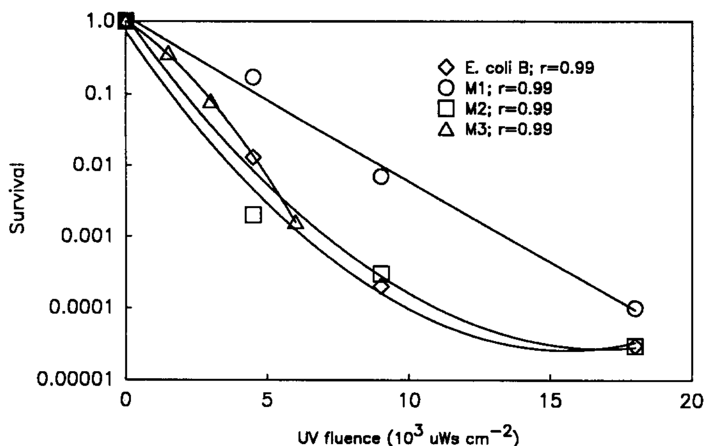

Fig. 2. Survival curves of *E. coli* B and three microaerophilic subsurface bacteria designated M1, M2 and M3 exposed to 254 nm UV light. Total dose was a function of the fluence rate and time of exposure. The fluence rate was 450 μW cm^{-2} for all bacteria with the exception of strain M3 which was exposed to a fluence rate of 300 μW cm^{-2}.

[17]. The exponential killing of *D. radiodurans* by gamma radiation was only achieved at extremely high doses. The D_{37} of *D. radiodurans* to gamma radiation was 300 krads, almost 30 × more resistant than the aerobic subsurface strains (11 to 12 krads). The *E. coli* strain tested was also much more sensitive to gamma radiation than *D. radiodurans* (Fig. 3), with a D_{37} of only 3.6 krads (Table 2).

The aerobic subsurface isolates were significantly more resistant to gamma radiation than were the microaerophilic isolates ($p < 0.05$). There was a shoulder at low gamma doses for UV1 but not for the other two aerobic isolates (Fig. 4). All three microaerophilic isolates had similar survival curves (Fig. 5). There was linear killing

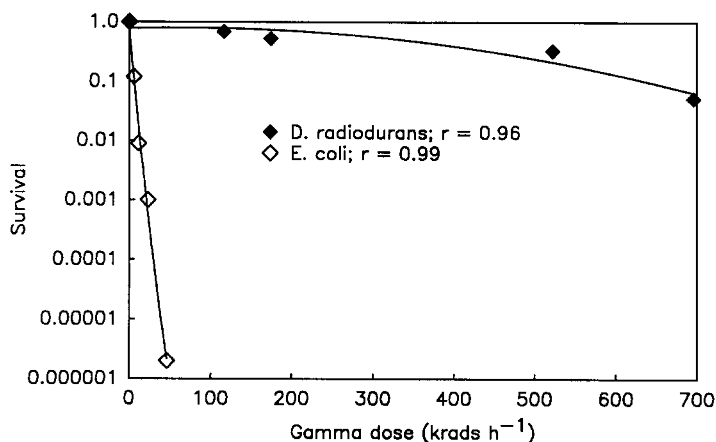

Fig. 3. Survival curves of *D. radiodurans* and *E. coli* ATCC 25922 exposed to a ^{60}Co source of 350 krads h^{-1}.

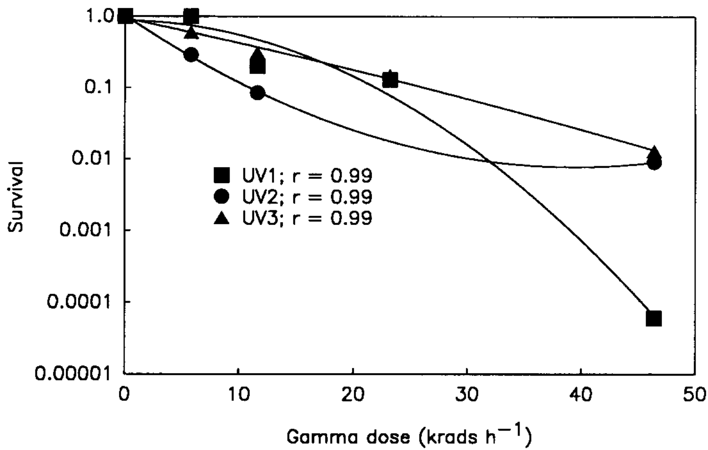

Fig. 4. Survival curves of the aerobic subsurface bacteria UV1–3 exposed to a ^{60}Co source of 350 krads h^{-1}.

with no indication of a shoulder region. The D_{37} values, 2.2–2.4 krads, were lower than those of the aerobic isolates.

Comparisons between UV light and gamma radiation

The effects of ionizing radiation and UV radiation on the survival of organisms are often similar [12,17]. Both radiation types induce similar lesions to the DNA double helix. The same major enzymatic mechanisms are utilized to repair UV and gamma radiation damage. Both radiation types can induce DNA strand breakage and pyrimidine dimer formation. However, while pyrimidine dimers are the major cause of cell lethality from UV light; the failure to repair strand breakage is the cause of cell

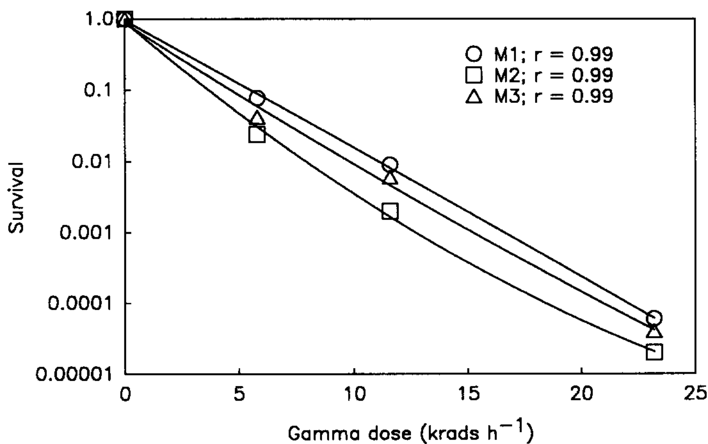

Fig. 5. Survival curves of the microaerophilic subsurface bacteria M1–3 exposed to a ^{60}Co source of 350 krads h^{-1}.

death from ionizing radiation [17]. Therefore, the survival kinetics of an organism exposed to each treatment may differ.

The similarity of resistance to UV and gamma radiation was valid in the subsurface bacteria tested. The UV resistant Gram positive aerobic isolates were also more resistant to gamma rays than the Gram negative microaerophiles (Table 2). However, some differences were noted. The survival slopes were steeper when cells were exposed to UV light as compared to gamma rays. Also, the shouldered regions of the survival curves of the aerobic subsurface bacteria when exposed to gamma irradiation were smaller than those for UV exposure. This appeared to indicate that the repair mechanisms of these bacteria were not as efficient in eliminating lesions induced by low doses of ionizing radiation as with low doses of UV radiation.

Although there were minor differences in the patterns of bacterial survival when the UV and gamma radiation treatments were compared, the order of resistance between the bacterial groups tested were distinct. *D. radiodurans* demonstrated the greatest resistance to both radiation types, while the aerobic Gram positive bacteria isolated from subsurface sediments were significantly more resistant to both radiation treatments than the microaerophiles. However the subsurface, microaerophilic strains had *D*-values similar to the reference *E. coli* strains known to be UV repair competent [19].

There is significant potential for the biological modification of mixed wastes in the subsurface environment [1]. However, the microorganisms utilized for biologically modifying radioactive waste must be able to tolerate the chemical and radioactive hazards that can exist at these sites. Because of the similarities of the bacterial responses between UV and ionizing radiation, it is proposed that UV light could be utilized to model bacterial survival to ionizing radiation. Bacterial strains could be screened for radiation resistance in a relatively short period of time without the necessity of accessing a gamma radiation source; thus reducing cost as well as exposure to radiation hazards.

Acknowledgements

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