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Survival of Subsurface Microorganisms Exposed to UV Radiation and Hydrogen Peroxide

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Aerobic and microaerophilic subsurface bacteria were screened for resistance to UV light. Contrary to the hypothesis that subsurface bacteria should be sensitive to UV light, the organisms studied exhibited resistance levels as efficient as those of surface bacteria. A total of 31% of the aerobic subsurface isolates were UV resistant, compared with 26% of the surface soil bacteria that were tested. Several aerobic, gram-positive, pigmented, subsurface isolates exhibited greater resistance to UV light than all of the reference bacterial strains tested except *Deinococcus radiodurans*. None of the microaerophilic, gram-negative, nonpigmented, subsurface isolates were UV resistant; however, these isolates exhibited levels of sensitivity similar to those of the gram-negative reference bacteria *Escherichia coli* B and *Pseudomonas fluorescens*. Photoreactivation activity was detected in three subsurface isolates, and strain UV3 exhibited a more efficient mechanism than *E. coli* B. The peroxide resistance of four subsurface isolates was also examined. The aerobic subsurface bacteria resistant to UV light tolerated higher levels of H_2O_2 than the microaerophilic organisms. The conservation of DNA repair pathways in subsurface microorganisms may be important in maintaining DNA integrity and in protecting the organisms against chemical insults, such as oxygen radicals, during periods of slow growth.

Tolerance to UV radiation has been related to the solar UV levels present in an organism's natural habitat (7, 16). Also, human- or animal-associated bacteria, such as *Legionella pneumophila* (1) and *Escherichia coli* (21), are generally more sensitive to far-UV light than other environmental bacterial isolates. Stamm and Charon (28) demonstrated that the free-living bacterium *Leptospira biflexa* was more resistant to far-UV light than *Leptospira interrogans*, which is associated with mammalian kidneys. UV tolerance is mediated by enzymatic repair of DNA damage through several well-documented pathways (12, 15, 29, 32, 33, 35), although overall UV resistance may also depend on physiological and behavioral traits, such as cell morphology, pigmentation, and phototaxis (7, 14, 16, 21, 36).

Microbiological investigations of subsurface environments have revealed the presence of diverse communities of metabolically active microorganisms. Balkwill and Ghiorse (4) reported total bacterial counts of up to 10^6 cells g^{-1} (dry weight) of soil in samples from saturated subsurface zones in Oklahoma, and Colwell (9) found a predominance of aerobic gram-positive bacteria in desert soil samples obtained from a depth of ca. 70 m. The findings obtained from drill sites in the eastern coastal plain, part of the Department of Energy Subsurface Science Program, included detection of aerobic (3, 8, 25), microaerophilic (5), and anaerobic (18) bacteria at depths of >400 m. Phelps et al. (25) determined that microbial activities in the eastern coastal plain sediments were highest in the water-saturated subsurface sands and lowest in the relatively impermeable clay zones. Bacterial isolates obtained from these soil enrichment cultures were capable of degrading a wide range of organic compounds, including hydrocarbons and aromatic and chlorinated aliphatic compounds, such as trichloroethylene (10).

The metabolic activities of deep subsurface microbial communities represent a potential source for bioremediation of terrestrial sediments and groundwater aquifers contaminated by organic and radioactive wastes (31). To succeed, in situ subsurface microorganisms may have to tolerate toxic levels of organic pollutants and/or sources of radiation. Although many of the genetic mechanisms involved in DNA repair are virtually ubiquitous in bacteria (e.g., *recA* [22]) and there is extensive regulatory overlap of DNA repair processes and other stress-induced responses, such as oxidative protection (13), there is wide variation in the phenotypic expression of UV radiation survival among different bacterial species and strains (21).

To determine to what extent DNA repair mechanisms have been conserved in subsurface microorganisms, subsurface bacterial isolates obtained from the Department of Energy Subsurface Science Program were examined for tolerance to DNA damage mediated by far-UV light (254 nm) and hydrogen peroxide. The results of recent studies have suggested that these subsurface isolates may have been isolated from surface effects after sedimentation, approximately 66 × 10^6 to 100 × 10^6 years ago (17, 26). Our hypothesis was that subsurface bacteria would be very sensitive to UV radiation because of the length of time that they have been separated from solar light. Aerobic and microaerophilic subsurface bacterial isolates and aerobic surface soil bacterial isolates were tested for UV resistance. Resistant isolates were grouped on the basis of their Gram reactions and the presence or absence of pigmentation. The UV survival curves for the two groups of subsurface isolates were compared with the survival curves for reference strains of surface bacteria to determine relative levels of UV tolerance. In addition, four subsurface bacterial isolates were examined for the presence of photoreactivating activity and resistance to the chemical toxicant H_2O_2.

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TABLE 1. Compositions of DSMS working stock solutions[a]

Compound	Concn (mg/liter of distilled water)
Mineral salt solution	
$NaNO_3$	9
NH_4Cl	55
$MgSO_4 \cdot 7H_2O$	50
$Na_2HPO_4 \cdot 12H_2O$	180
KH_2PO_4	68
$CaCl_2 \cdot 2H_2O$	5
Trace metal solution[b]	
Nitrilotriacetic acid	1,500
$FeSO_4 \cdot 7H_2O$	100
$MnCl_2 \cdot 4H_2O$	100
$CoCl_2 \cdot 6H_2O$	170
$ZnCl_2$	10
$CuCl_2 \cdot 2H_2O$	2
H_3BO_3	10
$Na_2MoO_4 \cdot 2H_2O$	10
NaCl	1,000
Na_2SeO_3	17
$NiCl_2 \cdot 6H_2O$	26
$Na_2WO_4 \cdot 2H_2O$	29
Vitamin solution	
Biotin	20
Folic acid	20
Pyridoxine-HCl	100
Thiamine-HCl	50
Riboflavin	50
Nicotinic acid	50
Panthothenic acid	50
Cyanocobalamin	1
p-Aminobenzoic acid	50
Lipoic acid	50

[a] DSMS medium contained 1 liter of the mineral salt solution, 3 ml of the trace metal solution, and 1 ml of the vitamin solution. DSMS medium also contained the following components (per liter [final volume]): 100 mg of MOPS (morpholinepropanesulfonic acid) buffer, 25 mg of glucose, 25 mg of peptone, 25 mg of tryptone, and 25 mg of yeast extract.

[b] Nitrilotriacetic acid was added to 200 ml of distilled water, and the pH was adjusted to 6.5 with KOH. Enough distilled water to bring the volume up to 1 liter was added, and the rest of the trace metal solution components were added in the order in which they appear on the table.

MATERIALS AND METHODS

Bacterial isolation, growth, and maintenance procedures. The subsurface bacterial strains used in this study were isolated from soil samples obtained from drill sites through the Subsurface Science Program of the Department of Energy (25). The primary isolation procedures used have been described previously (2). Briefly, subsurface soil from a depth of 150 to 500 m was inoculated into dilute-substrate mineral salts (DSMS) broth (Table 1), into DSMS semisolid medium tubes, or onto DSMS agar plates. Initial incubations at 25°C required more than 1 week before visible macroscopic growth was observed. Bacterial colonies were selected randomly, and the colonies selected included gram-positive and gram-negative, pigmented and nonpigmented, aerobic and microaerophilic isolates. Strains were judged to be microaerophilic on the basis of characteristic banding properties when they were inoculated into DSMS semisolid agar tubes. Most microaerophilic organisms also exhibited thin-film spreading motility on aerobically incubated DSMS agar plates (5). All subsurface isolates were maintained aerobically on DSMS agar plates or microaerophilically in semisolid DSMS medium tubes incubated at 25°C. A total of 39 aerobic and 24 microaerophilic subsurface bacterial isolates were tested for UV resistance.

Surface soil bacterial colonies were isolated from a Knoxville, Tenn., site. The soil samples were aseptically collected from a depth of 3 cm. The isolation and maintenance medium used for the surface soil isolates was DSMS agar. A total of 31 aerobic surface soil isolates, including gram-positive, gram-negative, pigmented, and nonpigmented colonies, were tested for UV resistance. No microaerophilic surface soil isolates were detected.

For all UV studies, subsurface and surface soil bacterial isolates were grown in modified DSMS broth supplemented with 10 times the normal amounts of glucose, peptone, tryptone, and yeast extract to increase the growth rate. With these levels of substrates, the subsurface isolates reached the stationary growth phase in approximately 48 h, compared with 1 week when normal DSMS media were used.

The reference bacterial strains used as controls in this study included *Deinococcus radiodurans* ATCC 13939, *Micrococcus luteus* ATCC 10240, *Pseudomonas fluorescens* ATCC 13525, and *Staphylococcus aureus* ATCC 12600. *Escherichia coli* B was obtained from the Biology Department, Virginia Polytechnic Institute and State University. All reference bacterial cultures were maintained at 30°C on tryptic soy agar plates (Difco Laboratories, Detroit, Mich.). The reference bacterial strains were grown in tryptic soy broth for testing.

UV irradiation procedures. The initial UV screening procedure was performed with stationary-phase cultures (24 h for reference bacteria; 48 h for the more slowly growing subsurface and surface soil isolates), which were washed and suspended in phosphate buffer and were manually agitated during irradiation as described previously (2). The UV fluence rate used in the initial screening procedure was 700 $\mu W\ cm^{-2}$, as determined with a UVX radiometer (UVP Inc., San Gabriel, Calif.). The total UV doses used in the initial screening procedure, as determined by the length of time that cultures were exposed to the fluence rate, ranged from 0 to 28,000 $\mu W\text{-}s\ cm^{-2}$. Fluence rates ranging from 150 to 700 $\mu W\ cm^{-2}$ were used to determine survival curves in later studies. There was no detectable deviation in the fluence rate over time. All UV irradiation procedures were performed under red light to prevent possible photoreactivation activity.

Following irradiation, a 1.0-ml aliquot of each suspension was serially diluted in buffer and spread plated. The plates were wrapped in foil and incubated in the dark for up to 10 days. The incubation temperatures were 25°C for soil isolates and 30°C for reference bacteria. The percentage of survival at each dose was determined by comparing the colony counts of irradiated cells with the colony counts of nonirradiated controls.

UV resistance was defined as a survival level of ≥1.0% at a UV dose of 14,000 $\mu W\text{-}s\ cm^{-2}$. At this dose, stationary-phase *E. coli* B cells exhibited a survival level of approximately 0.02% (data not shown).

Photoreactivation. Exponential-phase bacterial suspensions were UV irradiated as described above with the following modifications: aerobic subsurface isolates and the bacterium *D. radiodurans* were exposed to a UV fluence rate of 500 $\mu W\ cm^{-2}$, while microaerophilic subsurface isolates and *E. coli* B were exposed to a fluence rate of 300 $\mu W\ cm^{-2}$.

Immediately following irradiation, the bacterial suspensions were diluted, and duplicate sets of each bacterial culture were plated as described above. One set of plates was immediately wrapped in foil and incubated in the dark for up to 10 days. The second set of covered plates was

placed in a foil-lined box (24 by 46 by 24 cm) and exposed to a 365-nm photoreactivating light (PRL) source (Baxter Scientific Products, McGaw Park, Ill.) with a fluence rate of 200 μW cm^{-2}, as determined with a UVP radiometer. The exposure times to the PRL were 30 min for *D. radiodurans* and the aerobic subsurface isolates and 20 min for *E. coli* B and the microaerophilic subsurface isolates. The plates were incubated as described above, and the percentages of survival for each isolate with and without exposure to PRL were determined.

H_2O_2 challenge. Exponential-phase bacterial isolates were centrifuged, washed, and suspended in buffer as described above. A 1.27 M H_2O_2 working solution was made by diluting H_2O_2 (30% solution; Mallinckrodt, St. Louis, Mo.) with distilled deionized water; this solution was stored at 4°C in the dark. A fresh working solution was prepared for each experiment, and each working solution was less than 20 min old when it was used. Appropriate volumes of H_2O_2 to produce H_2O_2 concentrations of 0 to 640 mM were added to 15-ml screw-cap tubes containing media and 0.5-ml aliquots of a cell suspension (total volume, 5 ml). Subsurface isolates were tested in modified DSMS broth; *E. coli* B and *D. radiodurans* were tested in tryptic soy broth. Cells were challenged with each H_2O_2 dose for 15 min. Following exposure, 1.0-ml aliquots were diluted and plated as described above.

Calculations. D_{37}, D_{10}, and D_1 were defined as the fluence rates or concentrations of H_2O_2 which resulted in the reduction of the number of cells in a population to 37, 10, and 1% of the original number of cells, respectively. D_{37} and D_{10} values were calculated as described by Harm (12) in order to compare the sensitivities of unrelated bacterial strains to DNA-damaging treatments and to compare the results of this study with previously published values for the same bacterial species.

Certain postirradiation treatments, such as exposure to PRL, modify the effect of UV light on bacteria (12, 15, 29), and this change can be measured by a fluence reduction factor (FRF) (12). In this study FRF was defined as the ratio of the D_{37} value for UV-irradiated cells to the D_{37} value for irradiated cells exposed to PRL.

RESULTS AND DISCUSSION

Previously reported bacterial sensitivities to UV light vary. For example, previously published D_{37} values for *D. radiodurans* range from 35,000 to 60,000 μW-s cm^{-2} (24). The D_{37} for *D. radiodurans* obtained in this study (40,000 μW-s cm^{-2}) (Table 2) was in general agreement with these previously reported values. The D_{10} values for other reference bacterial strains obtained in this study were lower than those obtained by Meltzer and Rice (21); e.g., a D_{10} value for *M. luteus* of 9,700 μW-s cm^{-2} was determined in this study, compared with a D_{10} value of 19,700 μW-s cm^{-2} obtained by Meltzer and Rice. One explanation for the lower D_{10} values obtained in this study was the high UV fluence rates used. Harm (11) demonstrated that for *E. coli* B and B/r, a higher UV fluence rate (80 instead of 0.22 μW cm^{-2}) resulted in a decreased level of survival of irradiated cells. The fluence rates used in this study ranged from 150 to 700 μW cm^{-2}, compared with the fluence rate of 100 μW cm^{-2} used by Meltzer and Rice. These high fluence rates may have caused a greater rate of DNA damage accumulation, which saturated repair pathways and were manifested as increased sensitivity to the UV dose. However, the order of resistance to UV light for the reference bacterial species was generally the same (i.e., *D. radiodurans* was more resistant than *M. luteus*, which was more resistant than *S. aureus*, etc.).

TABLE 2. D_{37} and D_{10} values for bacterial isolates exposed to UV radiation

Organism(s)	No. of expt	D_{37} (μW-s cm^{-2}, 10^3)[a]	D_{10} (μW-s cm^{-2}, 10^3)[a]
D. radiodurans ATCC 13939	4	40 ± 13	91 ± 29
Aerobic subsurface isolates[b]	4	10 ± 1.8	16 ± 2.3
M. luteus ATCC 10240	3	5.0 ± 0.8	9.7 ± 0.7
S. aureus ATCC 12600	2	1.8 ± 0.35	4.1 ± 0.85
E. coli B[c]	2	1.7 ± 1.4	4.0 ± 0.35
Microaerophilic isolate[d]	3	1.7 ± 0.35	3.7 ± 1.2
P. fluorescens ATCC 13525	2	1.6 ± 0.07	3.6 ± 0.14

[a] D_{37} and D_{10} values were calculated from the regression lines of the exponential slopes of the survival curves as described by Harm (12).
[b] Includes isolates UV1, UV2, and UV3, which were the three most UV-resistant strains obtained from subsurface sediment samples.
[c] Obtained from the Biology Department, Virginia Polytechnic Institute and State University.
[d] Includes isolates M1, M2, and M3, which were representatives of the microaerophilic bacteria obtained from subsurface sediments.

UV resistance in subsurface bacteria. In our initial screening of subsurface isolates we concentrated on detecting bacteria that could tolerate extreme levels of UV radiation. All bacteria were tested for UV resistance during the stationary phase of growth since cells are most resistant to radiation at this stage (23, 30).

It has been suggested that resistance to near-UV light is dictated by the amount of solar radiation present in an organism's natural habitat (16), and previous correlations between habitat and near-UV (7) and far-UV (28) light have been observed. Therefore, low levels of tolerance to UV light by deep subsurface bacteria were expected, since these organisms have presumably been isolated from sunlight for millennia. Interestingly, the numbers of UV-resistant subsurface and surface soil bacterial isolates were similar (31 and 26%, respectively) (Table 3).

Although the possibility that microbial communities in these subsurface sediments have been contaminated with surface organisms over time cannot be ruled out, the results of both geologic and molecular studies do not support the hypothesis that there has been uniform and rapid transport of surface microorganisms to the deep subsurface environments. Sargent and Fliermans (26) noted that the interspersed clayey layers and upward hydraulic gradient of the area studied prevent the percolation of surface waters into deeper formations. Groundwater flow in the vicinity is such that transport from the recharge area to the deep aquifers

TABLE 3. Distribution of the UV resistance trait in stationary-phase, aerobic, soil bacteria[a]

Pigmentation	No. of surface isolates resistant to UV/ no. tested	No. of aerobic subsurface isolates resistant to UV/ no. tested
Pigmented	4/5	10/16
Nonpigmented	4/26	2/23
Total	8/31	12/39

[a] UV resistance was defined as a survival rate of ≥1.0% after exposure to 14,000 μW-s of 254-nm light per cm^2. The percentages of UV-resistant surface isolates and aerobic UV-resistant subsurface isolates that were gram positive were 62.5 and 83.3%, respectively. All microaerophilic, subsurface isolates were sensitive to UV light.

TABLE 4. D_{37} values for UV-irradiated bacteria that were subsequently exposed or not exposed to 365-nm PRL

Organism	D_{37} without PRL (μW-s cm^{-2}, 10^3)[a]	D_{37} with PRL (μW-s cm^{-2}, 10^3)[b]
D. radiodurans	37 ± 0.7[c]	38 ± 7.8
Aerobic subsurface isolates		
UV1	5.1 ± 0.4	4.8 ± 0.42
UV3	5.8 ± 0.35	18 ± 4.9
E. coli B	1.2 ± 0.07	2.2 ± 0.14
Microaerophilic subsurface isolates		
M1	1.6 ± 0.21	2.2 ± 0.28
M3	1.3 ± 0.07	2.0 ± 0.14

[a] The D_{37} values were calculated from the regression lines of the exponential slopes of the survival curves as described by Harm (12).

[b] The fluence rate of 365-nm PRL was 200 μW cm^{-2}. The aerobic subsurface bacteria and *D. radiodurans* were exposed for 30 min, while the microaerophilic subsurface bacteria and *E. coli* B were exposed for 20 min.

[c] Values are the means and standard deviations of the values from three replicate experiments.

takes on the order of thousands of years. There is also molecular evidence that suggests that limited movement of microorganisms in the subsurface occurs. Jimenez (17) examined the G+C contents and levels of DNA-DNA homology of 65 subsurface bacterial isolates obtained from various depths at three Department of Energy drill sites. While more than 60% of these isolates had G+C contents consistent with membership in the family *Pseudomonadaceae*, the DNA homology studies revealed that relatedness was based on depositional origin rather than depth or site location. These findings suggest that deep subsurface bacteria have had limited contact with surface organisms over time and may have been isolated for millennia.

The physiological characteristics of the UV-resistant bacteria were similar for the two sediment profiles examined with respect to pigmentation and Gram reaction. The majority of the pigmented bacteria in both the surface group and the subsurface group, including 10 of 16 subsurface pigmented strains and 4 of 5 surface soil pigmented strains, were UV resistant. Also, the two reference strains that were most resistant to UV radiation were the red-pigmented *D. radiodurans* strain and the yellow-pigmented *M. luteus* strain. The three subsurface bacteria that were most resistant to UV radiation (designated UV1, UV2, and UV3) possessed orange, yellow, and red pigments, respectively, and their average D_{37} was 10,000 μW-s cm^{-2} (Table 2). UV3, which was approximately twice as resistant to UV light as *M. luteus* (2), accounted for up to 5 to 10% of the total bacteria recovered from some deep subsurface soil samples containing 10^6 bacteria g^{-1} of soil (data not shown).

The relationship between pigmentation and protection from solar radiation has been observed previously. A colorless mutant of the halophile *Halobacterium cutirubrum* was more sensitive to UV light than the wild-type strain which possessed bacteriorhodopsin and bacterioruberin (36). Hermansson et al. (14) found significantly higher numbers of pigmented bacteria present at the air-water interface in marine coastal waters off the Swedish coast than in the bulk water column, which may indicate that there was a response to increased levels of solar flux at the water surface. Pigments have been implicated in near-UV radiation and free-radical protection, and the presence of carotenoids may protect against free-radical-induced cell membrane damage (34).

The majority of UV-resistant bacterial isolates obtained from both soil profiles were gram positive (62% of the resistant surface isolates and 83% of the resistant subsurface isolates). Among the reference strains tested, the gram-positive bacteria were generally more resistant to UV than the gram-negative organisms. It has been proposed by Jagger (16) that cell wall components may help deflect near-UV photons, resulting in a lower dose actually absorbed by target molecules in the cell. Therefore, it is possible that the thicker cell walls present in gram-positive bacteria screen a larger portion of UV light; thus, the amount of UV light reaching cellular DNA is decreased.

The aerobic, subsurface bacteria tested were similar to surface bacteria in that both UV-resistant and UV-sensitive isolates were present. In contrast to this heterogeneity, all 24 microaerophilic, subsurface isolates were UV sensitive, with the most resistant isolate exhibiting a D_1 of approximately 8,800 μW-s cm^{-2} (data not shown). In the only study in which the effects of UV light on a microaerophilic bacterium were investigated that we found, Butler et al. (6) reported that the 99.9% inactivation dose for *Campylobacter jejuni* was 1,800 μW-s cm^{-2}, indicating that this bacterium was extremely UV sensitive. Krieg and Hoffman (19) defined a microaerophile as an organism that is capable of oxygen-dependent growth, yet grows poorly or not at all in the presence of atmospheric levels of oxygen. Benoit and Phelps (5) found that subsurface microaerophilic bacteria prefer oxygen levels of 1 to 10% and often produce a sheen of growth at the surface and beneath the agar on aerobically incubated plates. However, while we detected no UV-resistant microaerophiles, the level of UV resistance exhibited by the microaerophilic isolates was similar to the level of UV resistance exhibited by the repair-competent organism *E. coli* B (also gram negative and nonpigmented) and greater than the D_1 value reported for *C. jejuni* (6).

Photoreactivation measurements. When *E. coli* B was exposed to PRL following UV irradiation, the D_{37} was 2,200 μW-s cm^{-2}, compared with a D_{37} of 1,200 μW-s cm^{-2} in the absence of PRL (FRF, 0.55) (Table 4). Therefore, UV light was less effective in inactivating the cell population exposed to PRL. The FRF for *D. radiodurans*, which does not possess a photoreactivating mechanism, was 0.97. Three of four subsurface bacterial isolates exhibited increased survival rates when they were exposed to PRL. The survival curves of subsurface isolates UV3 and M3 are shown in Fig. 1. Aerobic isolate UV3 exhibited a more efficient photoreactivating mechanism than *E. coli* B (FRF, 0.32). However, no photoreactivating effect was observed in the other aerobic, subsurface isolate, UV1 (FRF, ~1.0). Both microaerophilic isolates exhibited positive effects when they were exposed to PRL; the FRF were 0.73 and 0.65 for strains M1 and M3, respectively. However, the effect was not as significant as the effect observed with *E. coli* B or UV3. The photoreactivating enzyme (or photolyase) is constitutively produced in *E. coli* and is involved in the transcription and translation of one gene (12). Because of the length of time that subsurface bacteria have been screened from solar radiation, we hypothesized that these bacteria could have shed this gene and thus may not be able to photoreactivate DNA damage. However, our results favor the interpretation that this gene has been conserved in at least some subsurface bacteria.

Survival of bacteria exposed to H_2O_2. One explanation for the conservation of DNA repair in subsurface bacteria is that this mechanism is necessary for protection against chemical toxicity. The UV-resistant subsurface isolates UV1 and UV3

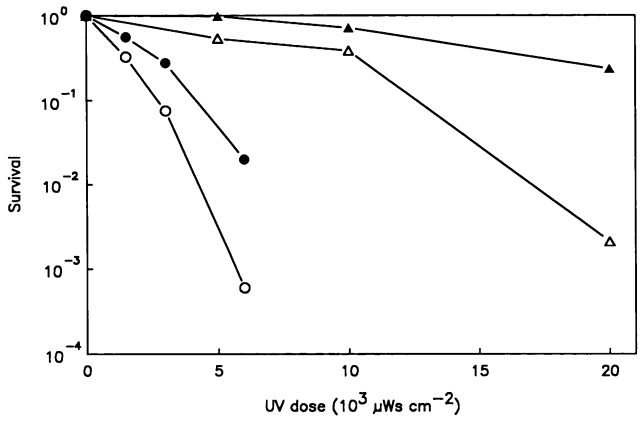

FIG. 1. UV survival curves for aerobic, subsurface bacterial strain UV3 and microaerophilic, subsurface bacterial strain M3 subsequently exposed or not exposed to 200 μW of 365-nm PRL per cm^2. Symbols: ▲, UV3 exposed to PRL; △, UV3 not exposed to PRL; ●, M3 exposed to PRL; ○, M3 not exposed to PRL.

exhibited higher tolerance to H_2O_2 than microaerophilic isolates M1 and M3 (Table 5). UV3 was more than six times as resistant to H_2O_2 than UV1 (D_{10} values, 290 and 47 mM H_2O_2, respectively). Microaerophilic isolates M1 and M3 produced survival curves with D_{10} values of 5.2 and 4.5 mM H_2O_2, respectively. Both UV1 and UV3 were more resistant to H_2O_2 than *D. radiodurans* (D_{10}, 33 mM H_2O_2) and *E. coli* B (D_{10}, 13 mM H_2O_2). Peroxide toxicity in bacteria is attributed in part to DNA damage (20). Although it is beyond the scope of this work, the similarity between UV resistance and peroxide resistance in subsurface bacteria may indicate that there are regulatory systems, such as the system mediated by the *rpoS* gene product in *E. coli*, which control the expression of genes involved in cellular protection from a variety of stresses, including radiation and H_2O_2 exposure (13).

The results of this study demonstrate that subsurface bacteria are as competent as surface bacteria in tolerating DNA damage induced by UV light and H_2O_2. This suggests that these subsurface bacteria have conserved DNA repair mechanisms despite the lack of exposure to solar radiation. In addition to the conservation of enzymatic DNA repair, there are several physiological characteristics that may contribute to overall DNA damage resistance, including pigmentation and cell wall thickness. The ability of some subsurface bacteria to survive high levels of radiation and chemical toxicants may represent a resource that can be exploited for in situ bioremediation of mixed organic and radioactive wastes in subsurface environments.

TABLE 5. D_{10} values for bacteria exposed to hydrogen peroxide

Organism	D_{10} (mM H_2O_2)[a]
D. radiodurans	33[b]
Aerobic subsurface isolates	
UV1	47
UV3	290
E. coli B	13
Microaerophilic subsurface isolates	
M1	5.2
M3	4.5

[a] The D_{10} values were calculated from the regression lines of the exponential slopes of the survival curves as described by Harm (12).
[b] Values are the means of the values from two replicate experiments.

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