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# Sensitivity of Selected Bacterial Species to UV Radiation

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**Abstract.** The effect of exposure of bacterial suspensions to UV radiation by means of the dose-response curves was assessed. The $D_{37}$ and $D_{10}$ values were used for subsequent statistical analysis of the results. The aim of this article is to evaluate the sensitivity to UV radiation of several microorganisms of different habitats (*Rhizobium meliloti*, *Rhodobacter sphaeroides*, *Escherichia coli*, and *Deinococcus radiodurans*), two mutants with nonfunctional SOS DNA repair system (*R. meliloti recA*$^-$ and *E. coli recA*$^-$), and a mutant in the synthesis of carotenoids (*R. sphaeroides crtD*). The results reveal that *D. radiodurans* was an extremely resistant bacterium, *R. meliloti* was more resistant than *R. sphaeroides*, and *E. coli* was the most sensitive bacterium tested. The high sensitivity of *recA*$^-$ mutants was also verify. Moreover, it seems that the possession of pigments had no important effect in the sensitivity of *R. sphaeroides* to UV radiation.

The solar radiation in the environment is potentially destructive [14]. Bacterial cells in nature are subject to DNA damage from exposure to solar radiation [12]. UV radiation is the main cause of the mutagenic and lethal effects of solar radiation. The role of *recA* in the repair of DNA suggests that this gene is largely responsible for the ability of bacteria to tolerate such damage and, hence, to exploit certain exposed ecosystems or regions of ecosystems [12]. Paradoxically, mutations, which are among the consequences of radiation, can have a selective advantage. From an evolutionary point of view, a balance between resistance and sensitivity to radiation could be beneficial, and it is perhaps for this reason that protection against radiation is normally not absolute. This resistance–sensitivity balance might be expected to differ from one organism to another. There is a wide diversity of resistance to both lethal and mutagenic effects of radiation. This diversity provides a considerable range for investigating the phenomena of radiation resistance and sensitivity.

It is commonly accepted that 3% of solar radiation is UV radiation (i.e., with a wavelength shorter than 390 nm), 37% is visible light (390–780 nm), and 60% is infrared (longer than 780 nm). As the Solar Constant is C = 1368.31 W m$^{-2}$ ± 0.05 [17], there is a value of 123.15 J m$^{-2}$ s$^{-1}$ that corresponds to UV radiation emitted by the sun. It has been estimated that only 0.00028% of solar radiation is significant for lethality in bacteria [15]. Therefore, $4 \times 10^{-3}$ J m$^{-2}$ s$^{-1}$ of lethal radiation reach the Earth's surface. The solar spectrum of UV radiation comprises three regions: ultraviolet A (UV-A), ultraviolet B (UV-B), and ultraviolet C (UV-C) radiation. UV-A radiation ranges from 390 to 320 nm, just beyond the violet portion of the visible spectrum. UV-B ranges from 320 to 286 nm, the latter wavelength being the shortest wavelength limit of sunlight at the Earth's surface. UV-C includes wavelengths shorter than 286 nm.

The incidence of UV radiation in the upper atmosphere causes the oxygen molecules present there to absorb all radiation under 200 nm and thus to change into ozone molecules. The ozone layer protects the surface of the Earth from biologically harmful UV radiation of wavelengths shorter than about 300 nm. For this reason, solar UV radiations below 300 nm are extremely scarce on the surface of the Earth. Only 2.2% of the solar radiation outside the atmosphere is associated with UV wavelengths below 320 nm, and 1% with UV wavelengths below 300 nm [2].

In this study, the resistance to UV radiation of seven bacterial strains of different habitats was examined (Table 1). *Rhizobium meliloti* was chosen as a

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Table 1. Some general characteristics of the strains used

| Strain | Source | Original habitat | Optimal temperature (°C) | mol% G + C of DNA (Tm) |
|---|---|---|---|---|
| *Escherichia coli* K-12 *recA*$^+$ | ATCC 23716 | human intestine | 37 | 48–52 |
| *Escherichia coli* HB101 *recA*$^-$ | UAB[a] | [c] | 37 | 48–52 |
| *Rhizobium meliloti* 2011 *recA*$^+$ | ATCC 9930 | soil | 25–30 | 62–63 |
| *Rhizobium meliloti* 2011 *recA*$^-$ | U. Biel.[b] | [c] | 25–30 | 62–63 |
| *Deinococcus radiodurans* | ATCC 13939 | unknown | 25–53 | 62–70 |
| *Rhodobacter sphaeroides* | ATCC 17023 | water | 30–34 | 70.8–73.2 |
| *Rhodobacter sphaeroides crtD* | UAB | water | 30–34 | 70.8–73.2 |

[a] UAB, Prof. J. Barbé, Autonomous University of Barcelona, Spain.
[b] U. Biel., Prof. A. Pühler, University of Bielefeld, Germany.
[c] Mutants obtained in the laboratory.

representative soil bacterium. We selected *Deinococcus radiodurans*, a ubiquitous bacterium, as an example of a bacterium highly resistant to UV radiation and to many other known mutagenic agents. The lack of information on the ecological distribution and natural associations of *Deinococcus* species adds to the difficulty of a significant taxonomic study. It seems that *D. radiodurans* may be widely distributed in nature in sites that include the human skin, although its numbers are modest. *Rhodobacter sphaeroides* was chosen as a good representative of phototrophic bacteria in aquatic environments. It is a facultative anoxygenic phototrophic bacterium, which contains bacteriochlorophyll *a* and spheroidene (a carotenoid pigment), and forms colonies with a characteristic purple color. A mutant in the synthesis of carotenoids (*R. sphaeroides crtD*) was also assayed. This mutant forms blue-green colonies because of a mutation in the spheroidene carotenoid biosynthetic pathway [10, 16]. We analyzed whether differences in pigmentation might result in different levels of resistance to radiation. Finally, *Escherichia coli*, which grows in the human intestine, was chosen because this microorganism is used for most genetic and physiological studies.

The RecA protein is directly involved in the repair of DNA lesions that result from UV irradiation. It participates in a recombinational process allowing lesion tolerance in damaged cells [12]. There is a level of radiation at which the number of DNA lesions will be lethal, preventing the cell from maintaining its metabolism at the minimal rate necessary for growth and reproduction. Therefore, quantification of cell survival can be used to evaluate biologically active UV radiation in a natural environment [5].

The exposure of *E. coli* to UV radiation induces a group of cellular functions called SOS functions [8, 12]. The induction of the SOS system depends on the activation of the *recA* gene product to a protease able to cleave the *lexA* gene product. The general repressor of SOS functions seems to contribute to the repair or processing of UV-induced DNA damage [9].

To demonstrate the range of sensitivity of these strains to irradiation, we worked with *recA*$^-$ mutants of *E. coli* and *R. meliloti*. The use of *recA*$^-$ mutant strains can be very useful in relation to the release of genetically engineering microorganisms (GEMs) to the environment.

## Materials and Methods

**Bacterial strains.** Table 1 shows the different strains used in this study, indicating the source and some distinctive characteristics of the strains.

**Growth conditions and culture procedures.** An overnight culture of each strain was grown at 30°C in Luria-Bertani (LB) nutrient medium under darkness conditions, with continuous agitation (200 rpm). Cells in the late exponential phase were taken. Depending on the final density of the culture, 1–3 ml was extracted. Care was taken to ensure that the final density of the suspension to be irradiated was not higher than $10^8$ cells ml$^{-1}$; otherwise, the survival curves would have been distorted, since UV radiation penetrates biological tissue poorly, and in a dense suspension of bacteria some cells are shielded by others [11].

To grow the two strains of *R.* sphaeroides, we used completely full 30-ml glass tubes. They were thus anaerobic cultures, and the bacteria had to grow phototrophically [10]. Five milliliter of the initial overnight cultures was introduced in each tube, and the tubes were filled with LB medium. Instead of minimal medium, LB medium was used to grow *R. sphaeroides* to avoid medium-related differences in growth. In the tubes a small air bubble (the size of a pea) was left to prevent the liquid pressure from increasing excessively because of temperature changes. The cultures were incubated under approximately 600 lux of white incandescent light (60 W) at 29°C, until they reached the desired cell concentration ($10^8$ cells ml$^{-1}$).

**UV irradiation procedure.** Each sample was centrifuged (5000 rpm for 10 min at 4°C), and the sediment was resuspended in 10 ml of Ringer ¼ solution. Afterwards it was spread onto a glass Petri dish (9 cm in diameter). Cell suspensions were irradiated with a UV lamp: 35 cm from a 15 watt low-pressure OSRAM HMS germicidal lamp with a peak of maximum emission at 254 nm, at an intensity of 1.7 J m$^{-2}$ s$^{-1}$ (determined with a Latarjet dosimeter). The time of exposure was different (between 5 and 240 s) in each strain, depending on the resistance to UV radiation. Unexposed samples (zero seconds) were used for control plates, which were packaged and handled in the same manner and at the same temperature as

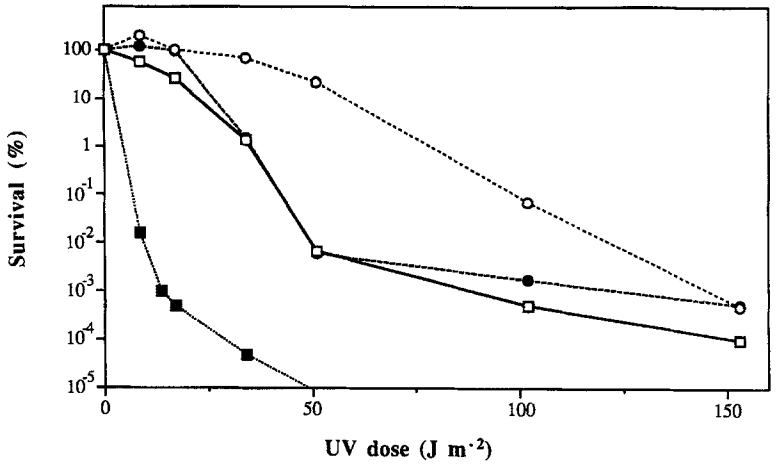


Fig. 1. Survival curves against UV radiation of *E. coli recA*$^+$ (□), *E. coli recA*$^-$ (■), *R. meliloti recA*$^+$ (○) and *R. meliloti recA*$^-$ (●).

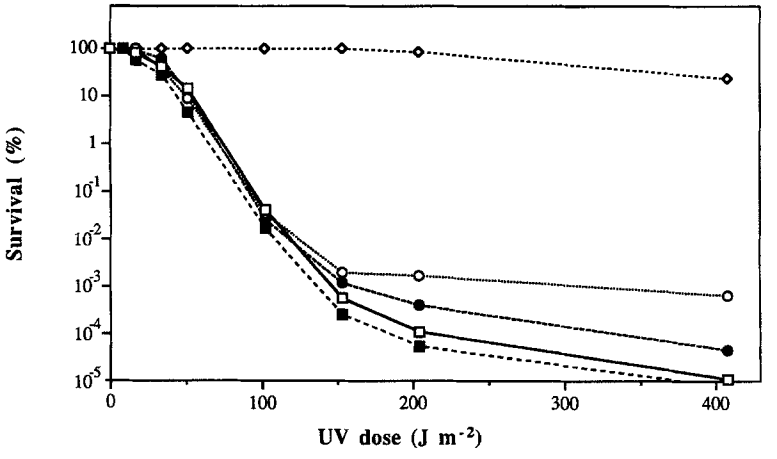


Fig. 2. Survival curves against UV radiation of *D. radiodurans* (◇), *R. sphaeroides* wild type in phototrophic (■) and heterotrophic growth (□), and *R. sphaeroides crtD* in phototrophic (●) and heterotrophic growth (○).

the exposed cell suspensions. Calculations of the percentage of cell survival after each treatment were made relative to the unexposed controls. The plates were grown in dark conditions to avoid photoreactivation mechanisms.

**Presentation of results.** Previous studies on resistance to UV radiation show only the dose–response curve. In this paper we try to assess the effect by using additional regression analysis to avoid the inaccuracy of the graph representation method.

We plotted the percentage of survival under UV radiation exposure in J $m^{-2}$ $s^{-1}$, (see Figs. 1 and 2). To compare the degrees of resistance of the bacterial strains with radiation treatments, $D_{37}$ and $D_{10}$ values were calculated. Sensitivity to radiation is often described by the $D_{37}$ value [13], 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. These D-values and the slopes of the survival curves are shown in Table 2.

## Results

The survival curves of two bacterial types, *E. coli* and *R. meliloti*, are represented in Fig. 1. To show the importance of their repair systems, the results of the *recA*$^+$ and *recA*$^-$ types of *E. coli* and *R. meliloti* are also represented in Fig. 1. As was expected, the *recA*$^+$ types exposed to UV radiation are more resistant, as can be observed from $D_{37}$ and $D_{10}$ values.

The $D_{37}$ value of *R. meliloti recA*$^+$ was twice that of *R. meliloti recA*$^-$, and *E. coli recA*$^+$ was 25-fold more resistant to UV radiation than *E. coli recA*$^-$. In fact, *R. meliloti recA*$^+$ was the second most resistant strain in our study. However, there are certain differences between *E. coli* and *R. meliloti*. The latter strain type reveals a UV radiation resistance higher than the two strains of *E. coli* tested. If *R. sphaeroides* is grown in anaerobic conditions and in presence of light, the synthesis of pigments takes place. Figure 2 shows the survival curves of two strains of *R. sphaeroides*, the wild type and the *crtD* mutant in both phototrophic and heterotrophic growth conditions. The survival curve of *D. radiodurans* is also shown. The survival curve for *D. radiodurans* exhibited a very gradual slope of −0.003 (Table 2) within the range of UV doses tested. This resulted in $D_{37}$ and $D_{10}$ values of 338.0 J $m^{-2}$ and 553.1 J $m^{-2}$ respectively. It is obvious

Table 2. D-values[a] (J $m^{-2}$) and slopes[b] from survival curves to UV

| Organisms | Slope | r | $D_{37}$ | $D_{10}$ |
|---|---|---|---|---|
| *Deinococcus radiodurans* | −0.003 ± 0.001 | −0.9998 | 338.0 ± 5.3 | 553.1 ± 8.7 |
| *Rhizobium meliloti recA*$^+$ | −0.044 ± 0.010 | −0.9987 | 42.4 ± 2.1 | 55.2 ± 2.7 |
| *Rhodobacter sphaeroides* wt (ht)[c] | −0.042 ± 0.015 | −0.9971 | 37.0 ± 2.8 | 50.5 ± 3.8 |
| *Rhodobacter sphaeroides crtD* (ph) | −0.041 ± 0.029 | −0.9889 | 36.4 ± 5.4 | 50.4 ± 7.5 |
| *Rhodobacter sphaeroides crtD* (ht) | −0.037 ± 0.025 | −0.9897 | 31.9 ± 4.5 | 47.2 ± 6.7 |
| *Rhodobacter sphaeroides* wt (ph) | −0.043 ± 0.014 | −0.9975 | 29.4 ± 2.1 | 42.7 ± 3.0 |
| *Rhizobium meliloti recA*$^-$ | −0.124 ± 0.014 | −0.9965 | 21.2 ± 1.8 | 25.8 ± 2.2 |
| *Escherichia coli recA*$^+$ | −0.106 ± 0.024 | −0.9869 | 17.3 ± 2.8 | 22.6 ± 3.6 |
| *Escherichia coli recA*$^-$ | −0.376 ± 0.031 | −0.9891 | 0.7 ± 0.1 | 2.2 ± 0.3 |

[a] D-values are 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 (D comes from dosis, see Methods).

[b] Slope calculated from the exponential portion of the survival curve (see Methods).

[c] wt, wild type; (ht), heterotrophic growth; (ph), phototrophic growth.

that *D. radiodurans*, as expected, was the most resistant bacterium examined. Only doses higher than 150 J $m^{-2}$ caused a slight decrease in the *D. radiodurans* survival curve.

The *R. sphaeroides* wild type and the *crtD* mutant showed similar behavior at low levels of UV radiation. However, when the exposure increased, the *crtD* mutant was more resistant than the wild type. We found that the *crtD* mutant grown in heterotrophic conditions was more resistant than the wild type of *R. sphaeroides*, and the same happened with the types that had grown phototrophically. Furthermore, when the two types of *R. sphaeroides* (the wild type and the *crtD* mutant) grew without oxygen (phototrophic growth), they were less resistant than when grown with oxygen (heterotrophic growth).

## Discussion

Most survival curves published are based on laboratory exposures to a fixed light source so that flux (fluence rate) and spectral quality are constant. In this situation, cellular responses follow a logarithmic curve, which is modified according to the repair capabilities of the organism. The UV lamp system used does not simulate natural sunlight. This experiment was designed to verify the dose response of selected bacterial species to UV radiation that has a constant flux and fixed spectral output. Neither of these properties remains constant during natural sunlight exposure.

Because of the wavelength dependency of biological responses, equal values of total irradiance obtained from integration across wavelength ranges can have different biological consequences. For this reason, it is very difficult to assess the biological relevance of measured radiation.

Harm [4] has established that the survival of *E. coli* cells after exposure to solar radiation of wavelengths >360 nm is partially dependent on DNA repair. Therefore, these longer wavelengths produce mediated in a high fluence the same DNA lesions as shorter UV-B wavelengths. Besides DNA damage, non-UV-B wavelengths interfere with other cellular processes. So, in our work the drastic effect of UV radiation on *E. coli* was demonstrated (*E. coli* was the most sensitive bacterium among all strains tested).

Moreover, the most UV-resistant bacterium in this study was *D. radiodurans*. This bacterium is Gram-positive, red-pigmented, and exceptionally efficient repairing radiation-induced DNA lesions [13]. The $D_{37}$ value for *D. radiodurans* is a little lower than those previously reported [1]. Those authors found 400 J $m^{-2}$, working with cultures in the stationary phase, whereas we took cultures in the exponential phase. The hypothesis of Freedman and Bruce [3] suggested that the growth rate should affect radiation resistance of bacteria. These authors found increased resistance in *D. radiodurans* with a decreased growth rate. Thus, for *D. radiodurans*, the resistance to radiation is lower during the exponential phase than in the stationary phase [6].

Low UV-radiation doses in *R. meliloti* strains caused an increase in the surviving fraction. This may explain the high resistance of *R. meliloti recA*$^+$ (Fig. 1). *R. meliloti recA*$^-$ has this increase in the surviving fraction too, but it has inactivated the SOS system and, for this reason, is more sensitive to UV radiation.

The results we obtained with *R. meliloti recA*$^-$ might be useful for constructing new GEMs. This affirmation is based on the low resistance of *R. meliloti recA*$^-$ to UV radiation (3000-fold more sensitive than *R. meliloti recA*$^+$, under 50 J $m^{-2}$ dose). If

researchers used a *R. meliloti recA*$^-$ strain as GEMs to liberate to the environment, they could easily control it. Their sensitivity to UV radiation would be a control mechanism of dispersion and could limit the undesirable genetic transfer in nature.

As *D. radiodurans* and *R. meliloti* strains come from habitats with similar doses of natural radiation, it is important to look for explanations other than the pressure of the ecological patch or natural selection. In fact, species can vary enormously in their natural resistance to radiation. This variation exceeds by far that expected on the basis of differences in the mean levels of radiation to which organisms are exposed. There is little or no correlation between natural levels of exposure and species resistance. The reason for this variation is not clear, but extreme resistance is likely to be a consequence of other features of a species [14].

Although the low resistance of *E. coli* to UV radiation may be owing to its low mol% G + C of the DNA, this hypothesis cannot be supported for other strains such as *R. sphaeroides*, which is the richest mol% G + C of the DNA, but is more sensitive than *D. radiodurans* (between 10-fold and 15-fold more sensitive to UV radiation in its $D_{37}$ value). However, this does not imply that the correlation was not valid. *D. radiodurans* might have other mechanisms of resistance that allowed it to survive under high doses of UV radiation even if the mol% G + C of the DNA was not very high. One of these mechanisms would be polyploidy; *D. radiodurans* presents five genome copies in each nucleotide [13]. This makes it more resistant to radiation damage on the DNA than strains with one only copy in each nucleotide. Another mechanism could be the presence of pigments. Organisms resistant to radiation are usually intensively pigmented, and many species of aquatic organisms are also pigmented, containing UV-absorbing compounds, or may have a cell wall or other external covering that acts as a filter for UV radiation [5]. As illustrated by Moseley [13], the characteristic pigmentation of the wild type of *Deinococcus* did not seem to play a major role in its resistance. In related investigations, an increase in the concentration of pigment through an alteration in conditions of culture was found to have little effect on radiation resistance [7]. In the present study, we observe that *R. meliloti* , an unpigmented bacterium, presents more resistance to UV radiation than *R. sphaeroides*. Moreover, strains of *R. sphaeroides* with a high quantity of pigments (phototrophic growth) are more sensitive than strains with a low quantity of pigments (heterotrophic growth).

As has been explained, the behavior of two strains of *R. sphaeroides* grown under two different conditions is similar. According to a Student's t-test ($p < 0.01$), there are no statistical differences in the D-values calculated from survival curves (Table 2), but high doses of UV radiation reveal differences between them. The possible explanation that the strain of *R. sphaeroides crtD* was more resistant than the wild type could be the different pigmentation presented in two strains. The wild type of *R. sphaeroides* is red because of the presence of carotenoid pigments. The *crtD* strain with a mutation in the spheroidene carotenoids biosynthetic pathway [16] acquires blue-green coloration because of the high quantity of bacteriochlorophyll *a* and the presence of chloroxantin [10]. We can presume that the bacteriochlorophyll *a* conferred it a resistance to high doses of UV radiation (absorption spectrum in the wavelength of UV radiation, like protection from oxidizing radicals, etc.). However, in this case, it only explained the difference between the wild type and the *crtD* mutant strains but not between the two types of cultures.

In phototrophic growth it happened in this way. Because there is no inhibition owing to oxygen, the bacteria synthesize a high quantity of pigments. In heterotrophic growth, the production of pigments is decreased, and, for this reason, the strains should be less resistant. In our results we have observed the opposite. Bacteria that have had a heterotrophic growth and have a low quantity of pigments are more resistant. Of course, all of this is under high doses of UV radiation, because under low doses the two types of strains under any conditions behave in the same way.

From these results, it seems that the possession of pigments is not an important factor in the sensitivity of *R. sphaeroides* to UV radiation. However, the high content of bacteriochlorophyll *a* could be an explanation of the higher resistance of CrtD mutant than the wild type. To interpret the differences between two growth conditions, a different physiological explanation has to be sought.

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