

DEINOCOCCUS RADIODURANS - A MODEL ORGANISM FOR LIFE UNDER MARTIAN CONDITIONS

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ABSTRACT

Deinococcus radiodurans, a gram positive bacterium whose ability to survive extremely high damage to its DNA which is for example induced by genotoxic chemicals, ionizing radiation, UV radiation or desiccation is still not completely understood. Because of its radiation and desiccation resistance, this extremophile is a prime candidate for possibly surviving environmental conditions as they occur on Mars and during a hypothetical interplanetary space travel. So far just minor work has been done on the damaging effects of UV radiation, especially polychromatic UV radiation in the UVB and UVA range, and desiccation/vacuum exposure, which is known to induce DNA damages similar to radiation. Therefore, we started to further investigate the radiation resistance and the repair mechanisms of *Deinococcus radiodurans*, concentrating on the effect of polychromatic UV radiation and desiccation in the wild type strain and ionizing radiation and UV radiation sensitive mutants. The results of this ongoing project will be of great interest to questions concerning planetary protection, space craft disinfecting measures and the search for life on other earth-like planets in general.

1. Introduction

Life on Earth originated in a climate very different from that of today. At that time the flux of energy-rich solar UV radiation was substantially higher. Short wavelengths of UVB and UVC radiation could reach the surface of the Earth, because there was no oxygen in the atmosphere and the formation of a short wavelength-UV protecting ozone layer was not possible. With the evolution of photosynthesizing organisms the oxygen concentration began to rise and an ozone layer began to form in the stratosphere. This led to a shift in the spectrum of solar UV radiation reaching the Earth's surface comprising today only

of wavelengths of 290 nm and longer (Fig. 1) (Cockell and Horneck, 2001). During the ongoing biological evolution nearly every environmental niche on Earth became occupied by microorganisms, even so-called 'extreme' environments. Extremophilic organisms can be used as models for the assessment of the chances of survival on other planets or moons of our solar system, e.g. on Mars or the Jupiter moon Europa (Horneck *et al.*, 2001). Mars as well as Europa are characterized by a high intensity radiation field. The UV climate of Mars is comparable to that of the early Earth when the biological evolution has begun. Some microorganisms are known, that can withstand such a high radiation burden like that on the surface of Mars.

In this study we have chosen *Deinococcus radiodurans* as test organism, because it is the most prominent example of a highly radiation resistant bacterium. It is ubiquitous, apathogen, pigmented, non-motile, non-spore forming, gram positive and occurs mainly in tetrads. The complex genome is completely sequenced (White *et al.*, 1999; Makarova *et al.*, 2001). *D. radiodurans* exhibits unusually high resistances to many DNA damaging agents. Most studies focused on its tolerance of DNA damages induced by ionizing radiation, but also the effects of germicidal ultraviolet (UV) radiation (wavelength of 254 nm) and cross-linking agents have been investigated (Moseley, 1983). *D. radiodurans* is highly resistant against desiccation as well. This can also be attributed to its DNA damage tolerance (Mattimore and Battista, 1996), because it is known that during the process of dehydration, DNA damages similar to those induced by γ radiation, including DNA double strand breaks, are formed and accumulate in the cells.

Up to now there are only very few detailed investigations on the UV radiation response of *D. radiodurans* to monochromatic radiation of other wavelengths than 254 nm (Setlow and Boling,

1965) and to polychromatic UV radiation (Caimi and Eisenstark, 1986). Especially the response to

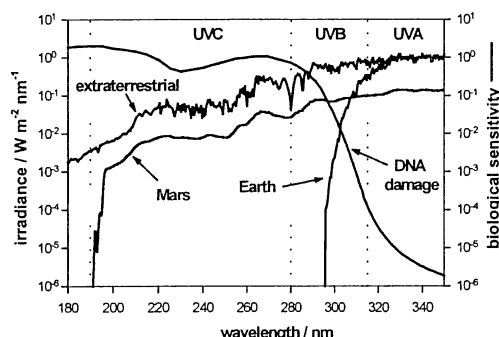


Fig. 1: Extraterrestrial solar spectral irradiance, spectral irradiance on Mars (calculated by M. Patel) and on Earth (left axis) and the DNA damage action spectrum (Setlow, 1974) (right axis)

the solar UV spectrum from 200 nm onwards corresponding to the present UV climate on Mars (see Fig. 1) is completely unknown. From experiments with combinations of monochromatic and polychromatic mars-like UV radiation, desiccation and low pressure conditions as damaging agents, informations will be obtained about the survivability of *D. radiodurans* on the surface of Mars. The results of this ongoing project will support the definition of planetary protection measures necessary to control the microbial contamination associated with space vehicles intended to land, flyby, or otherwise be in the vicinity of astrobiologically interesting extraterrestrial solar system bodies which have to be maintained as biological preserves for scientific investigations in the future.

2. Materials and methods

Bacterial strains: The following *D. radiodurans* strains were kindly provided by A. Vasilenko and M. J. Daly, Bethesda, MD, USA: R1 (wildtype), UV78 (*uvrA1*, *uvsE*), 1R1A (*recA*), IRS181 (*uvrA*⁺, *irrB*) and 262 (*uvrA2*).

Culture and irradiation conditions: The bacteria were cultivated in TYG medium (0.5 % Bacto-Trypton, 0.3 % Bacto-Yeast extract, 0.1 % D-

glucose) at 30 °C to the late exponential growth phase and washed 2x with phosphate buffered saline. The UV irradiations in suspension were performed additively in petri dishes (diameter 3.5 cm) with a cell concentration of 4×10^6 / ml and a volume of 3 ml with permanent stirring. For the UV irradiations of desiccated samples aliquots of 1×10^7 bacteria were dried on 7 mm quartz discs and stored at room temperature in a desiccator over anhydrous silica gel containing a visual indicator. After UV exposure the bacteria were resuspended and/or diluted, plated on TYG plates and incubated at 30 °C. The survival rate was determined from the ratio of the number of colony forming units of the irradiated samples to the unirradiated control samples of each experiment, N/N_0 .

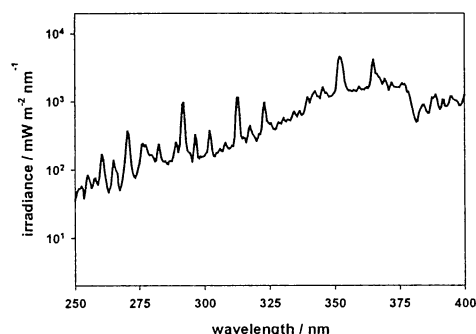


Fig. 2: Spectral irradiance of the solar simulator SOL2 without any filters

UV sources: For the characterization of the UV sensitivity of the *D. radiodurans* mutants a mercury low pressure lamp with the main UV emission line of 254 nm was used. Polychromatic UV radiation was produced with a solar simulator SOL2 (Dr. Hönle, Munich, Germany). The mars-like spectral irradiance of this lamp, measured with a spectroradiometer (Bentham 150, Gigahertz-Optics, Puchheim, Germany), is shown in Fig. 2. The dosimetry during each experiment was performed for both UV sources with a UVX radiometer (UVP Ultra-Violet Products, Cambridge, UK) with a 254 nm sensor head.

3. Results and discussion

The UV sensitivity of the 5 different *D. radiodurans* strains investigated in this study was verified by measuring the survival as colony

forming ability after application of monochromatic UV radiation of 254 nm, an UV standard wavelength used for sterilizing purposes, because the DNA, the most important target of UV radiation in every cell, has an absorption maximum nearby at 260 nm (see also Fig. 1). The resulting dose-effect curves for experiments in suspension are shown in Fig. 3. Strain 262 exhibits a similar UV resistance as the wildtype R1, whereas the strain IRS181 becomes more sensitive with UVC doses higher than about 800 Jm^{-2} . The strains UV78 and 1R1A are highly UV sensitive. These results are in good agreement with literature data (Moseley 1983; Upta et al., 1994; Gutman et al., 1994), where these strains have been characterized.

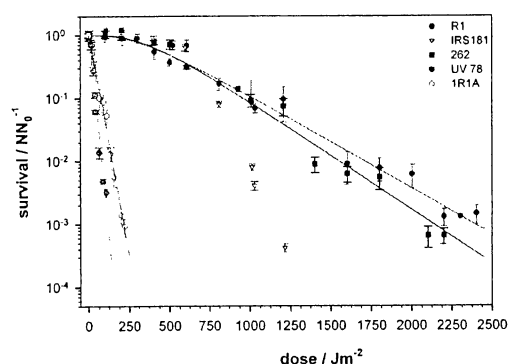


Fig. 3: Dose-effect curves of the different *D. radiodurans* strains after treatment with UVC radiation of 254 nm in suspension.

In Fig. 4 the corresponding dose-effect curves for the irradiation of the different strains in suspension with a mars-like spectrum of wavelengths > 200 nm are shown. Again the strain UV78 exhibits the highest UV sensitivity, whereas the difference between the more UV resistant strains 262, IRS181 and the wildtype R1 is smaller than with 254 nm. The ratio of the F_{37} , the dose, where each cell in a population has experienced on average one lethal event, with monochromatic UVC radiation to polychromatic UV radiation corresponds to 0.99 for R1, 0.97 for IRS181, 0.93 for 262, but 2.51 for UV78.

The desiccation resistance of the *D. radiodurans* strains was tested by storing air-dried bacteria in a desiccator at room temperature for different periods of time. In general, the titer of colony forming units decreases in the first 100 h of

desiccation and then remains constant at a level of about 60 % (e.g. for R1) and about 40 % (e.g. for UV78), respectively, up to about 1100 h of desiccation, the longest period tested in these experiments (data not shown).

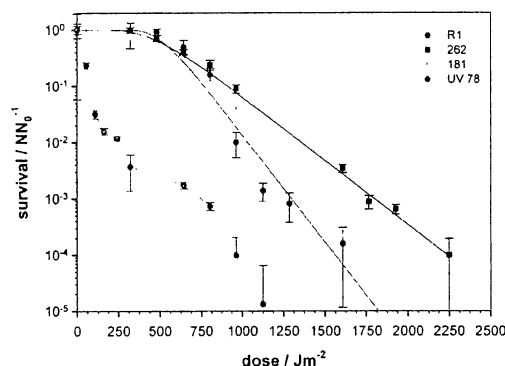


Fig.4: Dose-effect curves of the different *D. radiodurans* strains after treatment with mars-like UV radiation (> 200 nm) in suspension.

The effect of desiccation on the UV sensitivity of *D. radiodurans* is demonstrated in Fig. 5 and 6, where bacteria were first desiccated for 2 weeks and then exposed to UV radiation: to UVC radiation of 254 nm (Fig. 5) and to mars-like polychromatic UV radiation (> 200 nm) (Fig. 6). The UVC resistance of the wildtype R1 remains nearly unchanged after desiccation, whereas the resistance of the mutants increases and their dose-effect curves become exponential, in contrast to R1, still having a broad shoulder in the survival curve. The most remarkable increase in UVC resistance was found with the strain 1R1A, a *recA* mutant, where the UV resistance is about 10 times higher after desiccation ($F_{37} = 331 \text{ Jm}^{-2}$) compared to irradiation in suspension ($F_{37} = 33,6 \text{ Jm}^{-2}$). In contrast to this, the desiccation has only a very small influence on the UV resistance against polychromatic radiation. Only the form of the survival curves is different after desiccation, the shoulder decreases and the curves become more exponential.

From these results it can be concluded that the DNA damages, that are induced by desiccation, mainly doublestrand breaks (Mattimore and Battista, 1996), but also singlestrand breaks (no literature data available for *D. radiodurans*) are repaired after rehydration by induced enzymatic repair systems that are also able, at least to some extent, to repair UV-induced DNA damages. UV

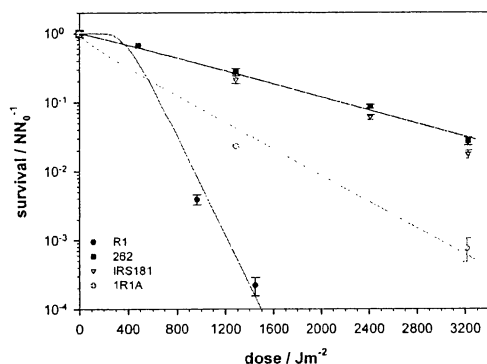


Fig. 5: Dose-effect curves of the different *D. radiodurans* strains after 2 weeks of desiccation and treatment with UVC radiation of 254 nm

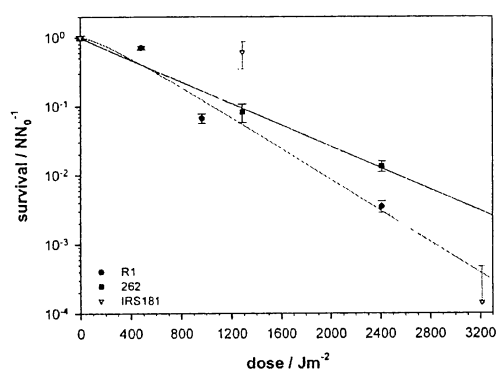


Fig. 6: Dose-effect curves of the different *D. radiodurans* strains after 2 weeks of desiccation and treatment with mars-like UV radiation (> 200 nm)

radiation produces mainly bulky lesions like pyrimidine-dimers, the most important photoproducts of UVC radiation, but also oxidative base damages in the case of longer UV wavelengths. For the different types of DNA photoproducts whole sets of enzymatic repair systems exist in *D. radiodurans* (White *et al.*, 1999; Makarova *et al.*, 2001). In the case of the wildtype strain R1 the synergistic action of the different, partly complementing DNA repair enzymes could not be observed, because of the simultaneous presence of all DNA repair systems and their overall very high efficiency. Especially the importance of the *recA* gene, responsible for the recombinational repair in many prokaryotes, and the complementing capacity of other

enzymatic repair systems could be demonstrated with the results of strain 1R1A (*recA*). Its UVC resistance becomes ten times higher after desiccation and reaches about two third of the level of the wildtype strain.

As next steps the detailed characterization of the UV- and desiccation-induced DNA damages on the molecular level, the investigation of the DNA repair kinetics and the determination of the survivability of *D. radiodurans* under mars-like conditions including temperature, low pressure and atmospheric composition are planned.

4. Conclusion

D. radiodurans's pronounced DNA damage tolerance based on complementing highly efficient enzymatic repair systems will presumably enable this bacterium to survive the 'extreme' environmental conditions on Mars. It can be used as a polyextremophile terrestrial 'worst case' model for the planning and testing of planetary protection measures for space craft sterilization procedures.

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