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A SIMPLE CONVENIENT BIOLOGICAL DOSIMETER FOR MONITORING SOLAR UV-B RADIATION

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SUMMARY. The use of dry *Bacillus subtilis* spores as a biological dosimeter for the monitoring of solar UV-B (290-330 nm) radiation was described. Our field tests had supported the utility of this dosimeter as a reproducible and reliable sunlight dosimeter.

A wide variety of biological systems are adversely affected by ultraviolet-B radiation (UV-B, 290-330 nm). Although this portion of the spectrum comprises only a part of the total solar energy reaching the surface of the earth, it is the most effective for producing biological effects where DNA is the target (1). Theoretical considerations suggest that a small reduction in the stratospheric ozone layer such as might be caused by supersonic aircraft emission, aerosol propellants and fertilizers may lead to an appreciable increase of solar UV-B radiation reaching the earth's surface. This has raised many concern as to the effects on ecological systems and human harzards such as skin cancer induction. Therefore, there is a need for monitoring solar spectral changes in the UV-B regions.

Although instruments are available and have been used for measurement of the sunlight spectrum in the UV-B region (2-5), the use of biological systems for long-term monitoring of solar UV-B radiation is scarce. In looking for a suitable biological system for sunlight dosimetry, Tyrrell (6) reported a comparative study using repair-deficient mutants from three biological systems and suggested the advantages of using repair defective *Bacillus subtilis* spores as a simple, reproducible biological dosimeter. To ease the handling of samples in transport and field experiments, Munakata further described the use of dry *B. subtilis* spores as a convenient biological sunlight dosimeter (7) and reported a long-term monitoring studies done at Tokyo (8).

In this communication, we describe another method of preparing spore sunlight dosimeter, employing also dry *B. subtilis* spores.

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MATERIALS AND METHODS

Bacterial strains and media. Spores of *B.subtilis* UVR (*thy met14*) and UVSSP-1-1 (*thy tryC2 uvrA10 ssp-1*) (9,10) were used in this work. The sproes of UVSSP-1-1 is defective in excision repair (*uvrA10*) and in the spore repair (*ssp-1*) for the removal of UV-radiation induced spore photoproducts (9,11). It is very sensitive to UV radiation killing, eg., the fluence at 254 nm that is required to inactivate 63% of UVSSP-1-1 spores is 1.5 J/m^2 as compared with 100 J/m^2 for spores of UVR. The media used for the determination of colony-forming units are Spizizen's minimal agar (12) supplemented with thymine at 10 μg/ml, L-alanine and required amino acids at 50 μg/ml. Schaeffer nutrient broth (13) is used for the production of spores.

Preparation of spores. Production of spores in Schaeffer nutrient broth has been described elsewhere (13). Spores were purified by centrifugation through a linear 50-100% Renografin-76 gradient (14).

The biological dosimeter. The dosimeter consisted of a piece of sterile, clean glass cover-slip (1.5 x 1.5 cm) which was glued onto a white S1624 pres-a-lay removable label (Dennison Co.). A ten-microliter aliquot of a mixed spore suspension containing 9 x 10^7 UVSSP-1-1 and 1 x 10^7 UVR spores per ml was placed in the center of glass cover-slip and allowed to dry over an area of about 1 cm^2 (monolayer). A layer of Saran wrap was then placed over the cover-slip and glued onto the pres-a-lay label to protect spores from contamination. A large number of such dosimeters can be easily prepared with very little cost and time. One layer of Saran wrap transmits about 90% of light in the wavelengths 290-330 nm.

Irradiation. (a) Monochromatic light. The source of monochromatic UV light (290-330 nm) was a Baush and Lomb grating monochromator (Model 33-86-45-49), illuminating with a 500 W high pressure mercury arc lamp (Philips SP-500). Fluence rates were measured with a Keithley 150B d.c. breaker amplifier and thermopile calibrated against a National Bureau of Standards carbon-filament standard lamp. The spores were irradiated at 1 x 10^7 cells/ml in water in a spoppered quartz cuvette with stirring. Samples were removed after different exposures, diluted appropriately and assayed for colony-forming units. **(b) Sunlight.** The dosimeters were placed on the roof-top of a building and exposed to sunlight for desired times. After exposure, they were kept in a dark box and brought back to the laboratory for assay as follows. The layer of Saran wrap was removed and 50 μl of sterile distilled water was placed over the cell paste on the cover-slip. After scratching off the spores into the water droplet, a 20 μl sample was removed into a test tube containing 1 ml of water. The cell suspension was diluted, and plated onto: (i) minimal agar containing thymine and tryptophan for the survivors of UVSSP-1-1 spores, and (ii) minimal agar containing thymine and methionine for the survivors of UVR spores. After incubation at 37° C for 2 days,

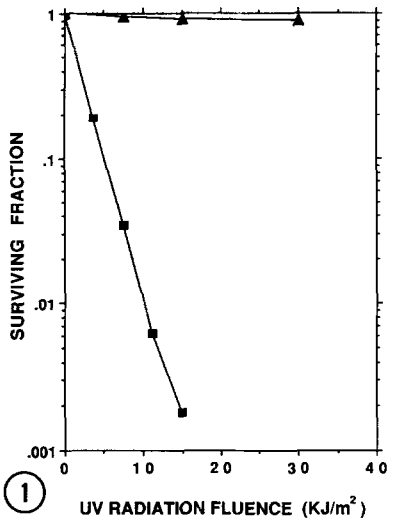
colonies were counted and the ratio of UVSSP-1-1 survivors to UVR survivors at each exposure time was determined. Since there is essentially no killing of UVR spores during the period of sunlight exposure (data not shown), the survival of UVSSP-1-1 spore can be calculated by dividing the ratios of UVSSP-1-1:UVR at each exposure time to the nonirradiated control. The inclusion of UVR spores in the dosimeter, therefore, serves as an internal control so as to eliminate any possible fluctuation in the recovery of dry spores from the cover-slip.

RESULTS AND DISCUSSION

The principal feature of our dosimeter is the inclusion of UVR spores as an internal control in the dosimeter to monitor the survival of repair-deficient UVSSP-1-1 spores after sunlight exposures. Its principle is illustrated in a test experiment in which a mixture of UVR and UVSSP-1-1 spores were exposed to monochromatic 313 nm radition and the survivors of each spore were determined by plating the irradiated spores onto selective media. As shown in Fig. 1, the UVSSP-1-1 spores were inactivated exponentially with increasing fluence, while the UVR spores were essentially not inactivated in the fluence ranges used. Since the UVR spores were not inactivated and could be differentiated from the UVSSP-1-1 spores in the spore mixture because of their different nutritional requirements, the survival of UVSSP-1-1 spore can also be calculated from the relative ratios of viable UVSSP-1-1 spores to UVR spores after different exposures to radiation.

To test the feasibility of our dosimeter for practical use, sets of triplicate dosimeters were exposed to sunlight on a clear day for 0-30 min, and were then brought back to the laboratory for assay as described in Materials and Methods. A typical result is shown in Fig. 2. The survival of UVSSP-1-1 spore was exponential with increasing time of exposure to sunlight. After 30 min of exposure to noon time sunlight at Dallas in May 1, 1977, the survival of UVSSP-1-1 spore was down to 6×10^{-3}. Therefore, within a reasonably short period of exposure to sunlght, the inactivation rate constant, 0.172 min^{-1}, for this mutant spore can be accurately determined. Knowing the inactivation rate constant for this mutant spore at a given reference wavelength, eg., 4.35×10^{-4} $J^{-1}m^2$ at 313 nm, one can also express the effective sunlight UV-B radiation fluence in term of its equivalent fluence at 313 nm.

In theory, the inactivation of the repair-deficient spores by sunlight may be described by the formula:
$-\ln S = \int A_{(\lambda)} I_{(\lambda)} d_\lambda$, where S is the surviving fraction, λ is wavelength, $A_{(\lambda)}$ is the wavelength dependent inactivation cross section, and $I_{(\lambda)}$ is the solar irradiance spectrum. The value of $A_{(\lambda)}$ can be determined experimentally from the action spectrum for the inactivation of this UV seneitive spore in the wavelength region 290-330 nm (data not shown) and is expressed as: $A_{(\lambda)} = 4.35 \times 10^{-4} \times e^{-(\lambda - 313/5.52)}$ $(J \cdot m^{-2})^{-1}$



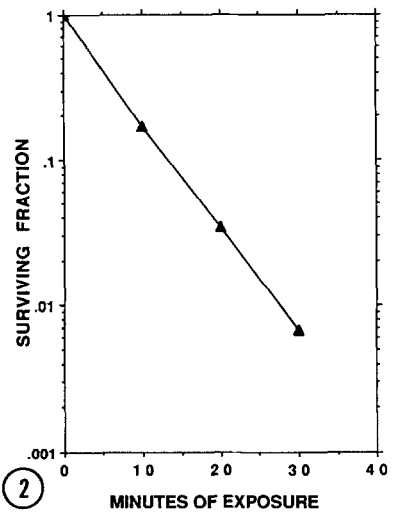


Figure 1. The survival curves of *B. subtilis* spores after exposure to 313 nm radiation. A mixture of UVR (▲) and UVSSP-1-1 (■) spores were irradiated with monochromatic 313 nm radiation and survivors were determined as described in Materials and Methods.

Figure 2. The survival curve of UVSSP-1-1 spore as a function of sunlight exposure. Sets of triplicate dosimeters were exposed to sunlight on the roof-top of a building from 12:30 to 13:00. They were brought back to laboratory and assayed for survivors as described in Materials and Methods. Each point was calculated from the average ratio as determined from the 3 dosimeters.

for UVSSP-1-1 spore, where λ is wavelength in nm. If we use Green et al.'s semi-empirical analytical formula (15) for the calculation of the solar UV-B, $I_{(\lambda)}$, reaching the earth's surface, the relative effects of solar spectrum for the inactivation of UVSSP-1-1 spore can be determined at each solar angle (Fig. 3). Our analyses indicate that the most effective solar wavelengths for the inactivation of this spore lie between 305 to 315 nm depending on the solar angles. In addition, if we graphically integrate these curves, we find that greater than 99% of the solar inactivation of this spore is provoked by wavelengths in the 290 to 340 nm region. This indicates that the biological dosimeter described here is principally monitoring solar UV-B radiation. Using a series of UV cut-off filters, Munakata (8) determined that the most effective solar wavelengths for sporocidal action are in the range of 308-325 nm, in agreement with the present analyses.

The advantages of using repair defective spores for sunlight dosimetry had been discussed (6). Exposure of spores to sunlight in suspension, however, has limited its applicability as a convenient dosimeter to carry around for field measurements. To circument this problem, Munakata employed spores dried on membrane filter and determined that spores dried on a membrane exhibited similar sensitivity as those suspended in water (7), thereby, greatly

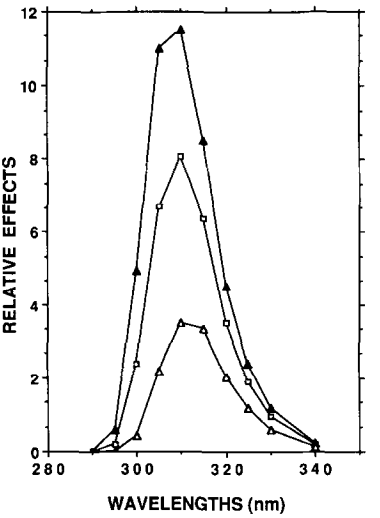


Figure 3. The relative effect of sunlight spectrum for the inactivation of UVSSP-1-1 spore at different solar angles. The curves were generated by multipling $A_{(\lambda)}$ with $I_{(\lambda)}$ as described in the Results and Discussion. The $I_{(\lambda)}$ was obtained from the semi-empirical analytical formula of Green et al. (15) for the ozone depth of 0.32 cm. The relative effect was expressed as arbituary units. Symbols at different solar angles: 90°, ▲ ; 60°, □ ; and 40°, △ .

widening the applicability of spore dosimeter for field measurements. One of the drawback in the spore dosimeter described by Munakata (7) was that the recovery of spores from the membrane may fluctuate from sample to sample which could affect the accuracy of assay. In the dosimeter described in this work, we have included UVR spores in the dosimeter to serve as an internal control. This should eliminate the recovery problem. However, one should aware that the applicability of the present dosimeter is based on the assumption that the UVR spore was essentially not inactivated while the UVS spore was inactivated down to 1% survival. Although this is true for most of the irradiation conditions, Munakata did observe that under certain heavy exposure conditions, there was appreciable inactivation of UVR spores as well (7). To minimize any complication as might arise from the inactivation of UVR spores, it is advised that the initial slope of inactivation curve be used for the determination of inactivation rate constant.

Another point that should be discussed is the use of Saran wrap in the present dosimeter. Although this wrap is fairly transparent to UV-B radiation, the transmittance at shorter UV wavelengths tends to be lower which could affect the spectrum of incident solar UV-B. Therefore, before we can find a UV transparent wrap to substitute the Saran wrap, it is advised that the Saran wrap be removed during the exposure of spore dosimeters to solar irradiation.

The UVSSP-1-1 spore used in the present work has the same DNA repair defect as the UVS(TJK6312) spore used by Munakata (7,8).

These two spores were expected to exhibit similar response to UV radiation. The inactivation rate constants in $J^{-1}m^2$ for UVSSP-1-1 spores were 0.67 and 0.00043 with 254 and 313 nm radiation, respectively. These values are slightly greater than the 0.5 and 0.0004 values reported for UVS(TJK6312) spores (7). Whether this difference is due to dosimetry or the bacterial strains used remains to be resolved.

The need of using biological systems for long term monitoring of solar UV-B radiation is apparent. At present, the spore system is the best characterized system and a long term monitoring study using dry spores has been reported (8). What we need more is to carry out similar long-term monitoring studies at various locations in the world. The present dosimeter is simple, accurate and reliable. This dosimeter should be an ideal choice for such long-term monitoring study.

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