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The Two Major Spore DNA Repair Pathways, Nucleotide Excision Repair and Spore Photoproduct Lyase, Are Sufficient for the Resistance of *Bacillus subtilis* Spores to Artificial UV-C and UV-B but Not to Solar Radiation

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Bacterial endospores are 1 to 2 orders of magnitude more resistant to 254-nm UV (UV-C) radiation than are exponentially growing cells of the same strain. This high UV resistance is due to two related phenomena: (i) DNA of dormant spores irradiated with 254-nm UV accumulates mainly a unique thymine dimer called the spore photoproduct (SP), and (ii) SP is corrected during spore germination by two major DNA repair pathways, nucleotide excision repair (NER) and an SP-specific enzyme called SP lyase. To date, it has been assumed that these two factors also account for resistance of bacterial spores to solar UV in the environment, despite the fact that sunlight at the Earth's surface consists of UV-B, UV-A, visible, and infrared wavelengths of approximately 290 nm and longer. To test this assumption, isogenic strains of *Bacillus subtilis* lacking either the NER or SP lyase DNA repair pathway were assayed for their relative resistance to radiation at a number of UV wavelengths, including UV-C (254 nm), UV-B (290 to 320 nm), full-spectrum sunlight, and sunlight from which the UV-B portion had been removed. For purposes of direct comparison, spore UV resistance levels were determined with respect to a calibrated biological dosimeter consisting of a mixture of wild-type spores and spores lacking both DNA repair systems. It was observed that the relative contributions of the two pathways to spore UV resistance change depending on the UV wavelengths used in a manner suggesting that spores irradiated with light at environmentally relevant UV wavelengths may accumulate significant amounts of one or more DNA photoproducts in addition to SP. Furthermore, it was noted that upon exposure to increasing wavelengths, wild-type spores decreased in their UV resistance from 33-fold (UV-C) to 12-fold (UV-B plus UV-A sunlight) to 6-fold (UV-A sunlight alone) more resistant than mutants lacking both DNA repair systems, suggesting that at increasing solar UV wavelengths, spores are inactivated either by DNA damage not reparable by the NER or SP lyase system, damage caused to photosensitive molecules other than DNA, or both.

Concerns about the potential harmful biological effects of a postulated increase in terrestrial UV flux due to stratospheric ozone destruction have in recent years sparked a considerable amount of experimental effort into investigating the effects of solar radiation on biological systems (reviewed in references 33 and 34). Of particular interest has been the study of mechanisms by which lethal and mutagenic damage is induced in cellular DNA by the UV portion of the solar spectrum as well as mechanisms used by living cells to repair or tolerate such damage (reviewed in reference 6). Bacterial endospores have been a particularly fruitful model system for studying the consequences of long-term exposure to environmental UV because of their simplicity and ease of use and transport, their stability during long-term storage, and the reproducibility of their inactivation response (30).

Most of the available information regarding the resistance of bacterial spores to UV has been derived from studies of the phenomenon in *Bacillus subtilis* because of the well-developed genetics and molecular biology available for this microorganism (38; reviewed in references 25 to 28). Dormant endospores of *B. subtilis* demonstrate high resistance to 254-nm radiation (UV-C) due to (i) accumulation in their DNA of the spore photoproduct (SP) 5-thyminyl-5,6-dihydrothymine (4, 35; reviewed in references 27 and 28) and (ii) accurate repair of SP during spore germination by two major DNA repair pathways, nucleotide excision repair (NER; encoded by genes designated *uvr*) and SP lyase (encoded by the *spl* gene) (5, 12, 17, 18). It appears that the NER and SP lyase pathways are also major determinants of spore resistance to solar radiation since mutant *B. subtilis* spores lacking both repair systems exhibit extreme sensitivity not only to laboratory UV-C radiation (12, 16) but also to the UV wavelengths present in sunlight (13–15, 20, 30, 36).

Results from previous studies using 254–nm UV and *B. subtilis* strains in which either NER or SP lyase had been inactivated by mutation indicated that SP lyase plays a more important role in determining spore UV-C resistance than does NER, since spores of *spl* mutants are more sensitive to UV-C than are spores of *uvr* mutants (5, 12). Solar radiation reaching the Earth's surface, however, is considerably more complex than artificially produced monochromatic 254-nm UV, consisting of a mixture of UV, visible, and infrared radiation, the UV portion spanning approximately 290 to 400 nm (the so-called UV-B and UV-A portions of the UV spectrum) (34). Therefore, as an attempt to relate current spore UV resistance models, built mainly on data from laboratory experiments using UV-C, to the resistance of spores to solar radiation in the environment, this communication describes experiments designed to assess the relative individual contributions

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TABLE 1. Bacterial strains used in this study

Strain (BGSC[a] code)	Genotype or phenotype	Source or reference[a,b]
B. subtilis		
168	*trpC2*	Lab stock
WN170	Trp^+ Cm^r	pWN162$\overset{tf}{\rightarrow}$168
WN171 (1A345)	*metC14 thyA1 thyB1 trpC2 sul uvrA42*	BGSC
WN173 (1A488)	*metC14 thyA1 thyB1 trpC2 sul spl-1*	BGSC
WN174	Same as WN173, but Trp^+ Cm^r	pWN162$\overset{tf}{\rightarrow}$WN173
WN175 (1A489)	*metC14 thyA1 thyB1 trpC2 sul spl-1 uvrA42*	BGSC
WN213	*trpC2* Δ(*ptsI-splAB*)::*ermC*	P. Fajardo-Cavazos
WN252	Same as WN175, but Met^+ MLS^r Δ(*ptsI-splAB*)::*ermC*	WN213$\overset{td}{\rightarrow}$WN175
E. coli		
(ECE11)	Strain MM294 carrying plasmid pTRP-H3	BGSC (1)
JM83	*ara* Δ(*lac-proAB*) *rpsL* ϕ80*lacZ*ΔM15	Lab stock (37)
WN162	Strain JM83 carrying plasmid pWN162	This study

[a] BGSC, *Bacillus* Genetic Stock Center.
[b] td, transduction; tf, transformation.

made by the NER and SP lyase DNA repair pathways to the resistance of *B. subtilis* spores to natural sunlight.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. The *B. subtilis* and *Escherichia coli* strains used in this study are listed in Table 1. Plasmid pWN162 carries a 3.6-kb *Hin*dIII fragment of DNA containing the *trpEDC* region from *B. subtilis* (1) cloned in the *Hin*dIII site of integrative plasmid pBGSC6. Plasmid pWN162 will transform *trpC2* mutants of *B. subtilis* to a Trp^+ Cm^r phenotype.

LB medium (11) was used for routine cultivation of *E. coli* and *B. subtilis* strains. For preparation of spores, *B. subtilis* strains were cultivated in either solid or liquid Difco sporulation medium (DSM) (22) supplemented with the appropriate selective antibiotic. The following antibiotics were supplied at the indicated final concentrations: ampicillin, 50 μg/ml; chloramphenicol, 3 μg/ml; or a combination of erythromycin (1 μg/ml) and lincomycin (25 μg/ml), hereafter referred to as MLS. Liquid cultures were incubated with vigorous aeration, and all cultivations were at 37°C. Spores were harvested, purified by lysozyme treatment and buffer washing as described previously (19), heat shocked (80°C, 10 min), and stored until use as suspensions of 10^9 to 10^{10} CFU/ml in water at 4°C protected from light.

Sample preparation. Spores were mixed in pairwise combinations (strain WN171 with strain WN174 at a 1:1 ratio or strain WN170 with strain WN252 at a 1:5 ratio) and diluted in water to a final concentration of approximately 10^8 CFU/ml before use. Three 10-μl aliquots of each resulting suspension (containing approximately 10^6 CFU per aliquot) were spotted onto a sterile microscope slide. The final diameter of each spot was approximately 1 cm. The slides were allowed to air dry in the dark at 37°C and stored protected from light until use. Although the slides were always used within a few days of preparation, a separate control experiment demonstrated no loss of spore viability upon prolonged storage at room temperature in the dark (approximately 3 months) (data not shown). Each microscope slide contained triplicate spore spots. Strains which were used in these experiments carried different antibiotic resistance markers (Table 1), so that the numbers of spores of each strain in any chosen pair could be quantitated either (i) by plating on DSM containing the appropriate selective antibiotic or (ii) when one strain of the pair carried no antibiotic resistance marker, by plating samples onto DSM either lacking or containing the selective antibiotic and then quantitating the antibiotic-sensitive strain by determining the difference in CFU on the two plates.

UV transmission spectra. Various materials were used in this study either as UV cutoff filters or as UV-transparent coverings. They included Saran Wrap (Dow Brands, Indianapolis, Ind.), polystyrene sheets constructed from petri dishes (Fisher Scientific, catalog no. 8-757-12), and 0.5-in.-thick (1 in. = 2.54 cm) plate glass obtained from a local glass supplier (The Edge of Art Glass, Ft. Worth, Tex.). Each of the materials described above was placed into the beam of a visible-UV spectrophotometer (Ultrospec III; LKB), and their UV transmittance spectra in the 200- to 400-nm range were obtained. The spectra are reported as the percent transmittance of the material at a given wavelength, normalized to its transmittance at 400 nm (Fig. 1).

UV radiation sources and UV dosimetry. UV-C radiation was provided by a commercial low-pressure mercury arc lamp (model UVGL-25; UV Products, San Gabriel, Calif.), which emits essentially monochromatic 254-nm radiation. The source of UV-B radiation was a commercial medium-pressure mercury arc lamp (model UVM-57; UV Products) which emits a spectrum of UV wavelengths spanning 280 to 320 nm, with an emission maximum at 302 nm. This lamp was further modified to limit the possible emission of shorter UV wavelengths by the placement of a polystyrene filter between the lamp and the sample; this polystyrene layer is opaque to UV wavelengths of 280 nm or less (Fig. 1). The doses of UV produced by these two artificial UV sources were measured with a UVX radiometer (UV Products) with the appropriate calibrated probe for UV-C or UV-B. Doses are reported in joules per square meter. Biological dosimetry is described in a separate section (see below).

UV irradiation of samples. Sample slides were mounted on cardboard and irradiated by different UV sources. The time of exposure was determined empirically and varied depending upon the bacterial strain used and the source of UV. Unirradiated control samples were treated identically to irradiated samples but were either kept in an opaque box or wrapped with a layer of aluminum foil during exposure.

(i) Artificial UV exposure. Samples were placed on a rotating platform (30 rpm) perpendicular to the UV source. The UV-C lamp was set at a height of 30 cm above the sample, resulting in a dose rate of approximately 0.23 $J/m^2/s$. The UV-B lamp was placed 10 cm above the sample, resulting in a dose rate of approximately 5.2 $J/m^2/s$. Before each experiment, the lamps were operated for at least 15 min to stabilize their UV output.

(ii) Sunlight exposure. For exposure to solar radiation, the experiments were conducted in two equal-sized cardboard boxes (31 by 24 by 4.5 cm); the top of each box was equipped with a window (28 by 21 cm). Samples were affixed to a cardboard platform raised to 0.5 cm below window level. When the samples were exposed to full-spectrum sunlight, a single layer of Saran Wrap, which transmits all solar UV wavelengths (Fig. 1), was placed over the window. To filter out the UV-B portion of sunlight, the window was covered with 0.5-in.-thick plate glass, which is opaque to UV wavelengths of 320 nm or less (Fig. 1).

The boxes were placed in a horizontal position on the roof of Medical Education Building No. 2 of the University of North Texas Health Science Center in Fort Worth. Exposures started at 10:00 a.m. local time and ended at 2:00 p.m. local time, which corresponds to 10:17 a.m. to 2:17 p.m. Central Standard Time or 11:17 a.m. to 3:17 p.m. Central Daylight Saving Time. All experiments in this study were performed between February and September 1995. Whenever possible, experiments were performed on cloudless or nearly cloudless days to maximize sunlight intensity. Nevertheless, to obtain complete survival curves for particularly UV-resistant strains, or when exposing spores to the UV-A portion of sunlight only, it was necessary to perform a single experiment over the course

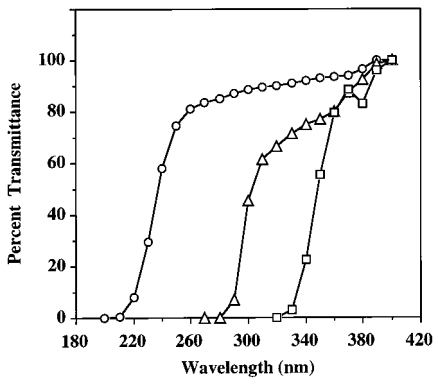

FIG. 1. UV transmittance spectra of materials used as filters in this study. Symbols: ○, Saran Wrap, single layer; △, polystyrene sheet; □, 0.5-in. plate glass.

of multiple (ranging from 2 to 8) days. In such cases, at the end of each daily exposure period, the sample boxes were covered with cardboard, moved to the laboratory, and stored in the dark until the next day when conditions were favorable for exposure to sunlight.

Recovery of spores and survival assay. After exposure of spores to UV or sunlight, samples were stored in an opaque slide box. Recovery of spores from the microscope slide was carried out by the method of Lindberg and Horneck (9). One hundred microliters of sterile 10% (wt/vol) polyvinyl alcohol (molecular weight, 30,000 to 70,000; Sigma Chemical Co., St. Louis, Mo.), prepared in water, was pipetted onto the surface of sample spots and air dried at 37°C for 1.0 to 1.5 h. Then, with a sterile scalpel and forceps, the polyvinyl alcohol film containing the spores was peeled from the slide. The polyvinyl alcohol film was dissolved in 1.0 ml of buffer (10 mM potassium phosphate [pH 7.4], 150 mM NaCl) and diluted serially 10-fold in the same buffer. Dilutions were plated on solid DSM plates containing the appropriate selective antibiotic, and the surviving fraction of the spores was determined by counting colonies after 20 to 40 h of incubation at 37°C (19). The survival percentage of spores (*%S*) was calculated by the following equation:

$$\%S = (N_t/N_0) \times 100 \quad (1)$$

where N_0 and N_t stand for the numbers of CFU of the spores at the exposure time t and time zero, respectively.

Biological dosimetry. The biologically effective dosage of UV radiation from sunlight or artificial UV sources was measured by use of the following test system, modified from Wang (36). Samples were prepared as described above with a mixture of spores of *B. subtilis* WN170 (wild-type; Cm^r) and WN252 (*uvrA42* Δ*ptsI-splAB*::*ermC* MLS^r). Spores of strain WN170 served as an internal control to eliminate fluctuations in the recovery of dry spores from the microscope slide, since there is essentially no killing of WN170 spores during the short periods of exposure used for biological dosimetry. Spores of strain WN252 are extremely sensitive to UV (4a), and their UV inactivation kinetics are strictly logarithmic (36). The percent survival of the dosimeter ($\%S_d$) was obtained from the equation:

$$\%S_d = (R_t/R_0) \times 100 \quad (2)$$

where R_t and R_0 stand for the ratio of WN252 CFU to WN170 CFU at time t and at time zero of exposure, respectively. The UV dosage or sunlight exposure time yielding 10% survival of the dosimeter (i.e., the 90% lethal dose [LD_{90}]) was defined as 1 sporicidal dosimeter unit (SDU).

Statistical analysis. LD_{90} values obtained from experiments testing spore resistance to artificial UV or sunlight were analyzed by analysis of variance. Values were analyzed in multigroup pairwise combination, and differences with a P value of less than 0.01 were considered statistically significant.

RESULTS

Control experiments. Several control experiments were performed to test the reliability and accuracy of both the spore dosimetry system and the protocols for recovering and quantitating survivors from the samples.

(i) Spore concentration-dependent shielding effects. It was previously reported that sample spots with too high a concentration of spores (5×10^6 CFU/3-mm-diameter spot) produce a shielding effect, thus resulting in an overestimate of spore UV resistance (15). Since there was some day-to-day variation in the concentration of spores applied to samples in our testing system (ranging between 6.0×10^5 and 1.4×10^6 CFU/10-mm-diameter spot), we tested strain WN170 spores for shielding effects over a range of 5.0×10^4 to 5.0×10^6 spores per sample spot. Spores of strain WN170 were exposed to different doses of UV-C, and survivors were quantitated. The results indicated that over the range of concentrations of spores used, there was no shielding (data not shown).

(ii) Heat resistance of spores. During the course of sunlight exposure experiments, it was observed that sample temperatures could approach 73°C at midday during summer. Control experiments were therefore performed to assess the effect of heat upon spore survival in the test system. For spores maintained at 60°C for 20 h, spore survival was still at least 70% (data not shown). In addition, no significant difference in heat resistance was observed among the four strains used in the experiments (data not shown).

(iii) Use of aluminum foil as a UV shield. To ensure that the sporicidal effects observed during sunlight exposure were due only to exposure to solar radiation, for each experiment a parallel set of spore samples was wrapped in one layer of aluminum foil and exposed in exactly the same manner as the test system. For every experiment and for spores of all strains tested, survival values at the end of the exposure period were above 70% for aluminum foil-shielded samples (data not shown). It should also be noted that sample temperatures during this control field experiment reached 73°C, similar to those used in the long-term heat resistance tests (see above), confirming that spores were stable under the high ambient temperatures prevailing during these experiments.

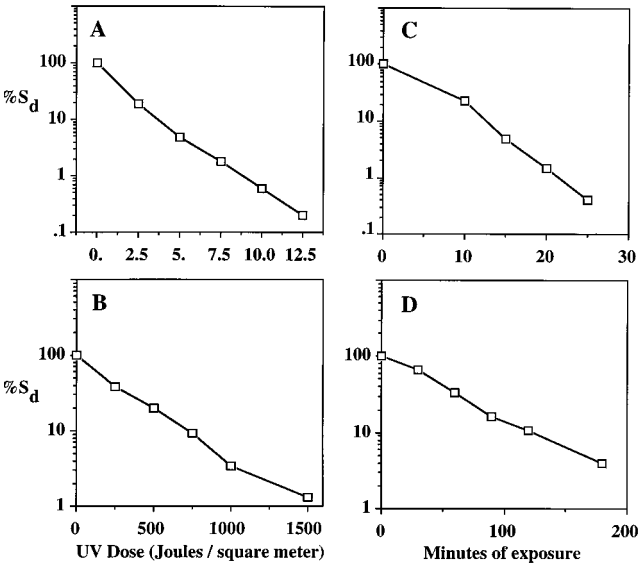

FIG. 2. Response of the spore dosimeter to UV-C (A), UV-B (B), full-spectrum sunlight (C), or sunlight from which UV-B had been filtered (D). (A and B) Averages of three independent experiments; (C and D) averages of two separate experiments performed on 4 August 1995 (C) and 3 September 1995 (D).

Measurement of sporicidal dosimeter response. In our dosimeter system, spores of *B. subtilis* WN252 and WN170 were mixed at a ratio of 5:1. The total spore concentration was kept near 1.0×10^6, which was determined to be free of shielding effects (see above) and which was used previously (36). To test the response of the dosimeter to UV, it was exposed to different sources of UV for various periods of time, and then spores were recovered and the percent survival was determined by using equation 2 (Fig. 2). Under artificially produced UV-C (Fig. 2A) or UV-B (Fig. 2B) radiation, the dosimeter was inactivated with exponential kinetics. When the dosimeter was exposed to full-spectrum sunlight (Fig. 2C) or to the UV-A portion of sunlight (Fig. 2D), a slight shoulder was observed in the survival curve of the dosimeter before exponential decline.

On the basis of dosimeter survival curves such as those shown in Fig. 2, LD_{90} values were determined for each exposure condition; to compare the biologically relevant dose received by the dosimeter with the physical UV-C or UV-B dose, the output of the artificial UV sources used was measured simultaneously. In response to artificially produced UV, 1 SDU corresponded to a dose of 3.45 J/m^2 for UV-C and 707.5 J/m^2 for UV-B (Fig. 2).

In the field, however, correlation of biological dosimeter response with physical dosimetry is more problematic because of the polychromatic nature of sunlight and the fact that solar radiation received at any given point on the Earth's surface is

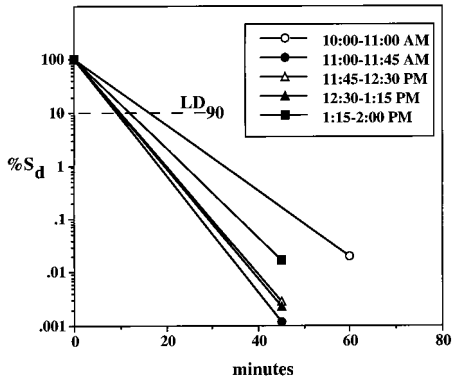

FIG. 3. Response of the spore dosimeter to full-spectrum sunlight, 10:00 a.m. to 2:00 p.m., 20 February 1995.

subject to constant variation due to fluctuations in atmospheric conditions and the continually changing solar angle. Therefore, in field experiments, the biologically relevant UV dose is usually defined in terms of the time in minutes for the dosimeter to accumulate 1 SDU during a given time interval (13, 36). For example, when exposed to full-spectrum sunlight on 4 August 1995, our dosimeter system measured 1 SDU in 12.6 min (Fig. 2C), and when exposed to sunlight from which UV-B had been filtered on 3 September 1995, the dosimeter measured 1 SDU after 118.3 min of exposure (Fig. 2D).

To calculate the total biologically relevant UV received by a spore sample over long-term UV exposure, it was necessary to measure the number of SDU registered by our dosimeter system over successive short intervals during the total exposure period and then total the values obtained. The results of dosimetry for a typical day (20 February 1995) are shown in Fig. 3. For example, when the dosimeter was exposed for a 60-min period from 10:00 a.m. to 11:00 a.m., 1 SDU of radiation was accumulated in 16.8 min; therefore, the dosimeter had been exposed to 3.57 SDU of sunlight during the 60-min exposure period (Fig. 3).

Resistance of spores to artificial UV-C and UV-B. To assess the relative contribution of NER or SP lyase to spore UV resistance, the levels of resistance of spores of strains WN170 (wild type), WN171 (*uvrA42*), and WN174 (*spl-1*) to artificially produced UV-C and UV-B radiation were determined, both in terms of relative SDU and absolute UV dose (Fig. 4). Upon UV-C irradiation (Fig. 4A), WN170 spores showed the highest resistance, followed by WN171 spores and then WN174 spores; this order of resistance was consistently obtained in each separate experiment. The LD_{90} values obtained were 33.0 SDU (113.7 J/m^2) for WN170, 17.0 SDU (58.6 J/m^2) for WN171, and 11.1 SDU (38.2 J/m^2) for WN174 spores (Fig. 4). Statistical analysis of the LD_{90} values obtained for WN170, WN171, and WN174 with UV-C indicated that the differences among the LD_{90} values for all three strains are significant at the 1% confidence level. Upon UV-B radiation (Fig. 4B), different results were obtained: WN170 spores still exhibited the highest resistance to UV-B, but the level of resistance of spores of strains WN171 and WN174 to UV-B were nearly identical. Upon several repetitions of the experiment, neither strain consistently demonstrated a higher level of resistance than the other. The average LD_{90} values obtained with UV-B were 31.4 SDU (2.2×10^4 J/m^2) for WN170, 15.4 SDU (1.1×10^4 J/m^2) for WN171, and 13.8 SDU (9.7×10^3 J/m^2) for WN174 spores (Fig. 4). Statistical analysis of the LD_{90} values obtained with UV-B indicated that there is no significant difference between

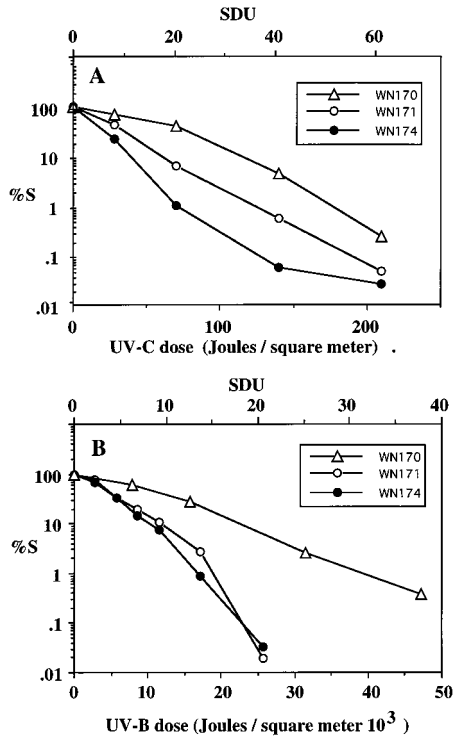

FIG. 4. Resistance of spores of strains WN170, WN171, and WN174 to artificially produced UV-C (A) or UV-B (B). The datum points represent the averages of two (WN170) or five (WN171 and WN174) separate experiments.

WN171 and WN174 at the 1% confidence level, although strain WN170 was significantly more UV-B resistant than either strain WN171 or WN174.

Resistance of spores to full-spectrum sunlight. To determine whether the laboratory model of spore UV-C resistance relates to spore resistance to solar radiation in the environment, we conducted experiments in which spores were exposed to natural sunlight.

The experiments were carried out from mid-February to mid-May 1995. Several experiments were done, each accompanied by dosimeter measurements and by control samples which were wrapped with a layer of aluminum foil during exposure. The results indicated that the observed pattern of survival to full-spectrum sunlight (Fig. 5A) resembled that seen with artificially produced UV-B radiation (Fig. 4B): WN170 spores had the highest resistance and the levels of resistance of WN171 and WN174 spores were similar, since upon several repetitions of the experiment, neither strain consistently demonstrated a higher level of resistance than the other. In response to full-spectrum sunlight, all three strains exhibited a shoulder on their survival curves before their exponential decline (Fig. 5A). The average LD_{90} values of strains WN170, WN171, and WN174 were 14.2, 8.9, and 9.3 SDU, respectively (Fig. 5A). Statistical analysis of the LD_{90} values between the three strains indicated that there was no significant difference between strains WN171 and WN174 at the 1% confidence level but that strain WN170 was significantly more resistant to full-spectrum sunlight than either WN171 or WN174.

Resistance of spores to sunlight lacking UV-B. The resistance of spores of strains WN170, WN171, and WN174 when exposed to the portion of sunlight containing only UV-A and longer wavelengths was then tested. This set of experiments was carried out from mid-June to mid-July 1995 in exactly the

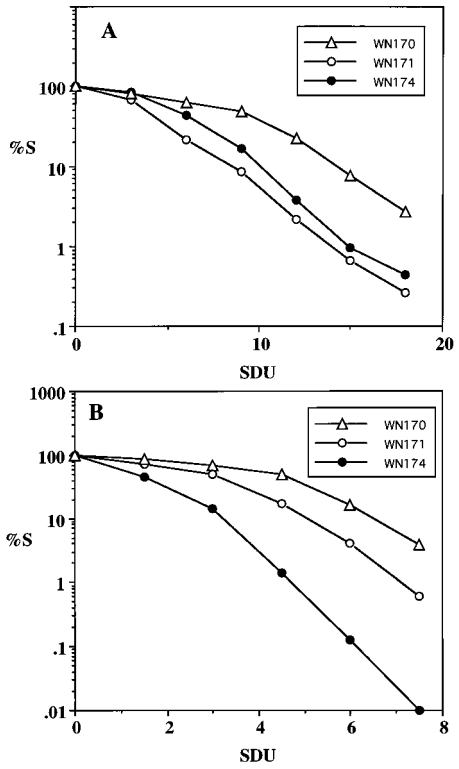

FIG. 5. Resistance of spores of strains WN170, WN171, and WN174 to full-spectrum sunlight (A) or sunlight from which UV-B had been removed (B). (A) The datum points represent the averages of four separate experiments performed between mid-February and mid-April 1995. (B) The datum points represent the averages of three separate experiments performed between mid-June and mid-July 1995.

same way as the full-spectrum sunlight exposure experiments, except that the UV-B portion of incident sunlight was removed by using 0.5-in.-thick plate glass as a filter. The results indicated that strain WN170 spores again had the highest resistance, followed by WN171 and then WN174 spores; the order of resistance was consistent over several experimental repetitions (Fig. 5B). As observed upon exposure of spores to full-spectrum sunlight (Fig. 5A), the survival curves of spores exposed to sunlight lacking UV-B also exhibited a shoulder before exponential decline (Fig. 5B). The calculated LD_{90} values for spores of strains WN170, WN171, and WN174 were 6.6, 4.6, and 3.2 SDU, respectively (Fig. 5B). Statistical analysis indicated that all three LD_{90} values were significantly different at the 1% confidence level.

DISCUSSION

A major factor in the long-term survival of bacterial spores in the environment is their ability to correct damage to their DNA induced as a result of their exposure to solar UV during dormancy. To date, it has been well established in the laboratory that SP which accumulates in the DNA of dormant spores irradiated with UV-C is corrected efficiently during spore germination through the combined activities of the NER and SP lyase repair pathways (12, 17, 18). It has also been established that these two DNA repair pathways working in concert are important for resistance of spores to sunlight (13–15, 20, 30, 36). In the present series of experiments, we have examined the relative individual contributions of NER and SP lyase to the

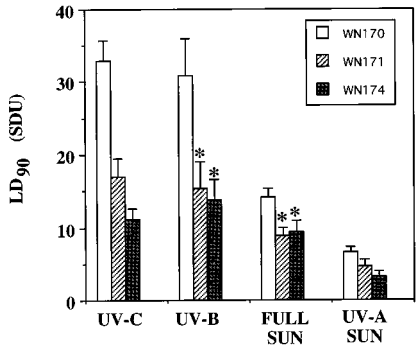

FIG. 6. Resistance of spores of *B. subtilis* WN170, WN171, and WN174 to various UV treatments. Treatments were artificial UV-C, UV-B, full-spectrum sunlight (full sun), and sunlight from which the UV-B component had been filtered (UV-A sun). The LD_{90} value of each strain is shown in SDU. Error bars denote standard deviations. Asterisks indicate no statistically significant difference between strains at the 1% confidence level.

survival of *B. subtilis* spores upon irradiation with artificially produced UV-C and UV-B as well as full-spectrum sunlight and the portion of sunlight containing UV-A and longer wavelengths. The experiments described in this communication were undertaken with a view towards testing the environmental relevance of the wealth of data which has been accumulated in the laboratory concerning spore UV-C resistance and spore DNA photochemistry. The results from these experiments reveal features of spore resistance to sunlight which the current laboratory model does not address.

Spore resistance to artificial UV-C versus UV-B. In response to UV-C, the results we obtained were in accord with previous experiments (12, 17, 18): wild-type spores were more than 30-fold more resistant than mutants lacking both NER and SP lyase, and SP lyase made a greater contribution to spore UV-C resistance than did NER (Fig. 6). Interestingly, however, when spore resistance to artificially produced UV-B was tested, wild-type spores were still 30-fold more resistant than spores lacking NER and SP lyase, but the relative contribution made by NER increased and that made by SP lyase decreased to the point at which the two repair systems made almost identical contributions to the final level of spore UV-B resistance (Fig. 6). These results suggest that spores exposed to UV-B wavelengths may accumulate one or more DNA photoproducts in addition to SP which can be preferentially repaired by the more nonspecific NER pathway. It appears that the use of single-mutant spores described in this communication results in a somewhat more wavelength-sensitive system than that of previous experiments. Working with a strain of *B. subtilis* which lacked both NER and SP lyase, Tyrell (30) postulated that an additional lethal DNA photoproduct was produced in spores by 365-nm UV-A, but he did not detect a difference in SP induced per lethal event in spores irradiated with 254-nm UV-C or 313-nm UV-B.

What is the identity of this postulated additional photoproduct? SP is clearly the principal DNA photoproduct formed in bacterial spores irradiated with UV-C (4, 35) and is also formed in major quantities in spores irradiated with artificial UV-B and UV-A as well as full-spectrum sunlight, although with a lower quantum efficiency at longer UV wavelengths (30). It has previously been demonstrated that *B. subtilis* spores lacking the major small, acid-soluble spore proteins (SASP) can accumulate a significant amount of cyclobutane-type pyrimidine dimers (PP) in addition to SP upon UV-C irradiation (23) and that spores of NER-deficient strains lacking SASP exhibit a more dramatic decrease in their UV-C resistance

than NER-deficient strains which contain SASP, presumably because of their inability to efficiently excise PP from DNA (24). Although PP is virtually undetectable in UV-C-irradiated bacterial spores (4, 23), it has not been systematically searched for in spores exposed to longer UV wavelengths and could be a possible candidate for a second spore DNA photoproduct. Also, other unidentified thymine-containing photoproducts in addition to SP have been detected in UV-C-irradiated spore DNA in minor quantities (4, 23). Evidence exists suggesting that one of these may be the pyrimidine-pyrimidone 6-4 photoproduct (10). The possibility exists that the action spectra of production of the 6-4 photoproduct or other minor photoproducts in spore DNA may be shifted towards longer UV wavelengths, resulting in their preferential formation in response to UV-B or sunlight (see below). In support of this notion, it has been observed that the distribution of cyclobutane dimers among CC, CT, and TT in UV-irradiated DNA is different, depending on whether UV-C or UV-B is used (29).

Spore resistance to full-spectrum sunlight. Exposure of spores to full-spectrum sunlight yielded a result resembling exposure of spores to artificial UV-B in that both NER and SP lyase pathways contributed roughly equally to spore resistance (Fig. 6); this finding again suggests the notion that full-spectrum sunlight may induce a DNA photoproduct(s) in addition to SP. The spore resistance patterns observed for NER-deficient or SP lyase-deficient mutants upon either UV-B or full-spectrum sunlight exposure appeared to be similar, probably because the most effective solar wavelengths for sporicidal action are in the UV-B region (305 to 325 nm) (14, 36). It is interesting, however, that wild-type spores were only 14-fold more resistant to full-spectrum sunlight than were spores of double mutants lacking both NER and SP lyase, in contrast to a 30-fold difference in the resistance of these strains to artificially produced UV-C or UV-B (Fig. 6), possibly because of factors discussed below.

Spore resistance to UV-A sunlight. Although much attention has focused on the UV-B portion of sunlight since it is a major cause of DNA damage, recent research has clearly demonstrated that UV-A, especially wavelengths in the 320- to 365-nm range, can also induce production of pyrimidine dimers besides producing other kinds of DNA and cellular damage (30; reviewed in reference 32). Several lines of evidence indicate that absorption of UV-A by cellular compounds can result in the generation of active oxygen intermediates, such as hydrogen peroxide or superoxide anion. These activated oxygen species can certainly damage the DNA molecule, yielding single-strand breaks or protein-DNA cross-links. Oxygen-dependent enzyme photoinactivation and lipid peroxidation are also examples of ways in which active oxygen species are capable of causing potentially lethal damage to other, non-DNA, cellular components (reviewed in reference 32).

In our test of the resistance of spores to solar radiation consisting of UV-A or longer wavelengths, it appeared that SP is still a DNA photoproduct, because SP lyase contributed more to total spore UV resistance than did NER (Fig. 6). However, wild-type spores were only 6-fold more resistant than double mutant spores to sunlight lacking UV-B, an even smaller difference than the 14-fold difference noted between these two strains with respect to resistance to full-spectrum sunlight (Fig. 6). The results strongly suggest that, in contrast to artificially produced UV-C or UV-B, solar radiation is capable of producing lethal damage to spores which is reparable by neither NER nor SP lyase and that these types of lethal damage are attributable particularly to wavelengths greater than 320 nm.

There are many other DNA repair systems which exist in *B. subtilis* besides NER and SP lyase, such as base excision repair, recombinational repair, and SOS-mediated repair systems (38). At present, it is unclear what contribution these repair systems make to spore resistance to solar radiation; certainly, the contribution of *recA*-mediated repair can be readily tested, since *B. subtilis recA* mutants are available. However, the target(s) of lethal solar damage to spores may not necessarily be restricted to DNA; recent studies indicate that bacteria possess overlapping inducible stress regulons which respond both to oxidative stress and to UV-A (7, 8, 21, 31) and that *B. subtilis* itself coordinates catalase synthesis via overlapping stationary-phase-inducible and oxidative-stress-inducible control circuitry (2, 3). Such findings suggest that the resistance of spores to solar UV is a complex and coordinated global stress response which challenges current laboratory-based models that address only UV-C photodamage of spore DNA and its repair.

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