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Roles of Small, Acid-Soluble Spore Proteins and Core Water Content in Survival of *Bacillus subtilis* Spores Exposed to Environmental Solar UV Radiation[▽]

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Spores of *Bacillus subtilis* contain a number of small, acid-soluble spore proteins (SASP) which comprise up to 20% of total spore core protein. The multiple α/β-type SASP have been shown to confer resistance to UV radiation, heat, peroxides, and other sporicidal treatments. In this study, SASP-defective mutants of *B. subtilis* and spores deficient in *dacB*, a mutation leading to an increased core water content, were used to study the relative contributions of SASP and increased core water content to spore resistance to germicidal 254-nm and simulated environmental UV exposure (280 to 400 nm, 290 to 400 nm, and 320 to 400 nm). Spores of strains carrying mutations in *sspA*, *sspB*, and both *sspA* and *sspB* (lacking the major SASP-α and/or SASP-β) were significantly more sensitive to 254-nm and all polychromatic UV exposures, whereas the UV resistance of spores of the *sspE* strain (lacking SASP-γ) was essentially identical to that of the wild type. Spores of the *dacB*-defective strain were as resistant to 254-nm UV-C radiation as wild-type spores. However, spores of the *dacB* strain were significantly more sensitive than wild-type spores to environmental UV treatments of >280 nm. Air-dried spores of the *dacB* mutant strain had a significantly higher water content than air-dried wild-type spores. Our results indicate that α/β-type SASP and decreased spore core water content play an essential role in spore resistance to environmentally relevant UV wavelengths whereas SASP-γ does not.

Spores of *Bacillus* spp. are highly resistant to inactivation by different physical stresses, such as toxic chemicals and biocidal agents, desiccation, pressure and temperature extremes, and high fluences of UV or ionizing radiation (reviewed in references 33, 34, and 48). Under stressful environmental conditions, cells of *Bacillus* spp. produce endospores that can stay dormant for extended periods. The reason for the high resistance of bacterial spores to environmental extremes lies in the structure of the spore. Spores possess thick layers of highly cross-linked coat proteins, a modified peptidoglycan spore cortex, a low core water content, and abundant intracellular constituents, such as the calcium chelate of dipicolinic acid and α/β-type small, acid-soluble spore proteins (α/β-type SASP), the last two of which protect spore DNA (6, 42, 46, 48, 52). DNA damage accumulated during spore dormancy is also efficiently repaired during spore germination (33, 47, 48). UV-induced DNA photoproducts are repaired by spore photoproduct lyase and nucleotide excision repair, DNA double-strand breaks (DSB) by nonhomologous end joining, and oxidative stress-induced apurinic/apyrimidinic (AP) sites by AP endonucleases and base excision repair (15, 26–29, 34, 43, 53, 57).

Monochromatic 254-nm UV radiation has been used as an efficient and cost-effective means of disinfecting surfaces, building air, and drinking water supplies (31). Commonly used test organisms for inactivation studies are bacterial spores, usually spores of *Bacillus subtilis*, due to their high degree of resistance to various sporicidal treatments, reproducible inactivation response, and safety (1, 8, 19, 31, 48). Depending on the *Bacillus* species analyzed, spores are 10 to 50 times more resistant than growing cells to 254-nm UV radiation. In addition, most of the laboratory studies of spore inactivation and radiation biology have been performed using monochromatic 254-nm UV radiation (33, 34). Although 254-nm UV-C radiation is a convenient germicidal treatment and relevant to disinfection procedures, results obtained by using 254-nm UV-C are not truly representative of results obtained using UV wavelengths that endospores encounter in their natural environments (34, 42, 50, 51, 59). However, sunlight reaching the Earth's surface is not monochromatic 254-nm radiation but a mixture of UV, visible, and infrared radiation, with the UV portion spanning approximately 290 to 400 nm (33, 34, 36). Thus, our knowledge of spore UV resistance has been constructed largely using a wavelength of UV radiation not normally reaching the Earth's surface, even though ample evidence exists that both DNA photochemistry and microbial responses to UV are strongly wavelength dependent (2, 30, 33, 36).

Of recent interest in our laboratories has been the exploration of factors that confer on *B. subtilis* spores resistance to environmentally relevant extreme conditions, particularly solar UV radiation and extreme desiccation (23, 28, 30, 34 36, 48, 52). It has been reported that α/β-type SASP but not SASP-γ play a major role in spore resistance to 254-nm UV-C radiation (20, 21) and to wet heat, dry heat, and oxidizing agents (48). In

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contrast, increased spore water content was reported to affect *B. subtilis* spore resistance to moist heat and hydrogen peroxide but not to 254-nm UV-C (12, 40, 48). However, the possible roles of SASP-α, -β, and -γ and core water content in spore resistance to environmentally relevant solar UV wavelengths have not been explored. Therefore, in this study, we have used *B. subtilis* strains carrying mutations in the *sspA*, *sspB*, *sspE*, *sspA* and *sspB*, or *dacB* gene to investigate the contributions of SASP and increased core water content to the resistance of *B. subtilis* spores to 254-nm UV-C and environmentally relevant polychromatic UV radiation encountered on Earth's surface.

MATERIALS AND METHODS

***Bacillus subtilis* spores, sporulation, purification, and sample preparation.** Endospores of six different isogenic *Bacillus subtilis* 168-derived strains were used in this work: PS832, a Trp^+ revertant of strain 168 (17) (wild-type strain); PS1899, lacking DacB, a D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 5*) (40); PS283, lacking SASP-α (20); PS338, lacking SASP-β (20); PS356, lacking SASP-α and -β (encoded by *sspA* and *sspB*) (17); and PS483, lacking SASP-γ (12). All strains except PS832 are resistant to chloramphenicol (5 μg/ml). Spores were obtained by cultivation at 37°C with vigorous aeration in double-strength liquid Schaeffer sporulation medium (44), under identical conditions for each strain, and the spores were purified and stored as described previously (22–27, 37). Spore preparations were free (>99%) of growing cells, germinated spores, and cell debris, as determined by phase-contrast microscopy.

Determination of spore water content. Samples of spores in water were prepared containing known numbers of spores, determined by direct counting in a Neubauer hemocytometer. Triplicate samples of spores were placed in preweighed aluminum weighing boats and allowed to air dry at 20°C, with 33% relative humidity (RH), and weighed daily on an analytical balance to constant weight, which was reached after 3 days. Aluminum boats were transferred to a 110°C oven, dried for 24 h, cooled to room temperature in a desiccator, and weighed daily to a constant weight. Percent water was calculated as follows: [(weight of spores air-dried at 20°C − weight of spores oven dried at 110°C)/weight of spores oven dried at 110°C] × 100 (9).

Spore exposure to mono- and polychromatic UV radiation. Wet spore samples consisted of suspensions of spores (1×10^6/ml) in water. Dry spore samples consisted of air dried (33% RH) spore monolayers (2×10^7 spores) immobilized on 7-mm-diameter quartz discs (Heraeus Quarzglas GmbH & Co. KG, Hanau, Germany) (26, 27, 41). Effects of mono- and polychromatic UV radiation on spores were determined as described previously (23, 27).

Survival assay. To recover spores from the quartz discs after UV exposure, spore monolayers were covered by 10% aqueous polyvinyl alcohol, and after drying, the spore-polyvinyl alcohol layer was removed and dissolved in water as described previously (14, 24, 26, 27). Spore survival was determined from appropriate dilutions in distilled water as colony-forming ability after incubation overnight at 37°C on nutrient broth agar plates (Difco, Detroit, MI) as described previously (14, 22–27).

Numerical and statistical analysis. The surviving fraction of *B. subtilis* spores was determined from the quotient N/N_0, where N is colony formers after UV irradiation and N_0 is colony formers without UV irradiation. To determine the curve parameters, the following relationship was used: $\ln N/N_0 = -IC \times F + n$, where IC is the inactivation constant (m^2/J), F is the applied UV fluence (J/m^2), and n is the extrapolation number. The IC was determined from the slope of the fluence effect curves as described by Moeller et al. (23). All data shown are expressed as averages ± standard deviations ($n = 3$ or 4). The results were compared statistically using Student's t test. Values were analyzed in multigroup pairwise combinations, and differences with P values of ≤0.05 were considered statistically significant (22–27).

RESULTS

Role of SASP and spore water content in spore UV resistance in aqueous suspension. In order to confirm previous results regarding resistance of wild-type, *dacB*, and *sspA sspB* mutant spores to 254-nm UV and to extend these results to environmentally relevant UV wavelengths, triplicate samples of spores in aqueous suspension were irradiated with UV of various wavelengths and survival kinetics determined. Spore resistance was then quantified by calculating the F_{10} value, the applied UV fluence leading to a spore survival of 10% for each strain, and the F_{10} values compared by Student's t test (Table 1). In response to 254-nm UV, the strains behaved as reported previously (20, 40); *dacB* spores were not significantly more sensitive than wild-type spores, and *sspA sspB* spores were significantly more sensitive than wild-type spores (Table 1). However, while *sspA sspB* spores were also significantly more sensitive to environmentally relevant UV wavelengths [280- to 400-nm and 290- to 400-nm UV-A + UV-B {UV-(A+B)} and 320- to 400-nm UV-A], *dacB* spores were also significantly more UV sensitive than were wild-type spores (Table 1), thus indicating a role for both α/β-type SASP and decreased spore water content in spore resistance to environmental UV when spores are in aqueous suspension.

TABLE 1. Survival characteristics of UV-irradiated *B. subtilis* spores in aqueous suspension[a]

Strain description	F_{10} value with treatment			
	UV-C, 254 nm ($J \cdot m^{-2}$)	UV-(A+B), 280–400 nm ($kJ \cdot m^{-2}$)	UV-(A+B), 290–400 nm ($kJ \cdot m^{-2}$)	UV-A, 320–400 nm ($kJ \cdot m^{-2}$)
Wild type	303 ± 25	10.1 ± 1.5	14.2 ± 1.6	498 ± 57
dacB	280 ± 23	7.7 ± 1.2*	8.1 ± 1.4*	252 ± 37*
sspA sspB	78 ± 11*	5.3 ± 0.7*	6.3 ± 0.8*	159 ± 26*

[a] Data are averages and standard deviations ($n = 3$). Asterisks indicate survival values that were significantly different from those for wild-type spores ($P \leq 0.05$).

Role of SASP and spore water content in air-dried spore UV resistance to 254-nm UV-C radiation. Because germicidal (254-nm) UV is often used to disinfect dry surfaces and building air (18, 31), it was of interest to understand the UV resistance of spores in the air-dried state. In order to extend results from previous studies of spore resistance to 254-nm UV in aqueous suspension, quadruplicate samples of the isogenic air-dried spores were exposed to 254-nm UV-C and spore inactivation kinetics plotted and fitted to exponential best-fit lines (Fig. 1A). Again, F_{10} values were calculated and compared using Student's t test (Table 2). When the data for 254-nm UV-C were analyzed, it was observed that *dacB* and *sspE* spores were not significantly more sensitive to 254-nm UV-C than were wild-type spores (Table 2) but that spores deficient in SASP-α, SASP-β, or both were significantly more sensitive to 254-nm UV-C radiation than the wild type (Table 2). Therefore, when air-dried spores of mutant strains were compared to wild-type spores, their relative sensitivities to 254-nm UV in the air-dried state were qualitatively comparable to results seen previously with spores in aqueous suspension (12, 20, 40) (Table 1).

Role of SASP and increased spore core water content in air-dried spore resistance to environmental UV radiation. Spores are often exposed to UV in the environment in an air-dried form on surfaces or adhered to soil or dust particles (32, 59). Therefore, we examined the contribution that various SASP or DacB made to spore resistance to simulated natural UV radiation wavelengths: 280 to 400 nm (UV-B plus UV-A), 290 to 400 nm (simulating the UV-B plus UV-A radiation reaching Earth's surface), and 320 to 400 nm (UV-A).

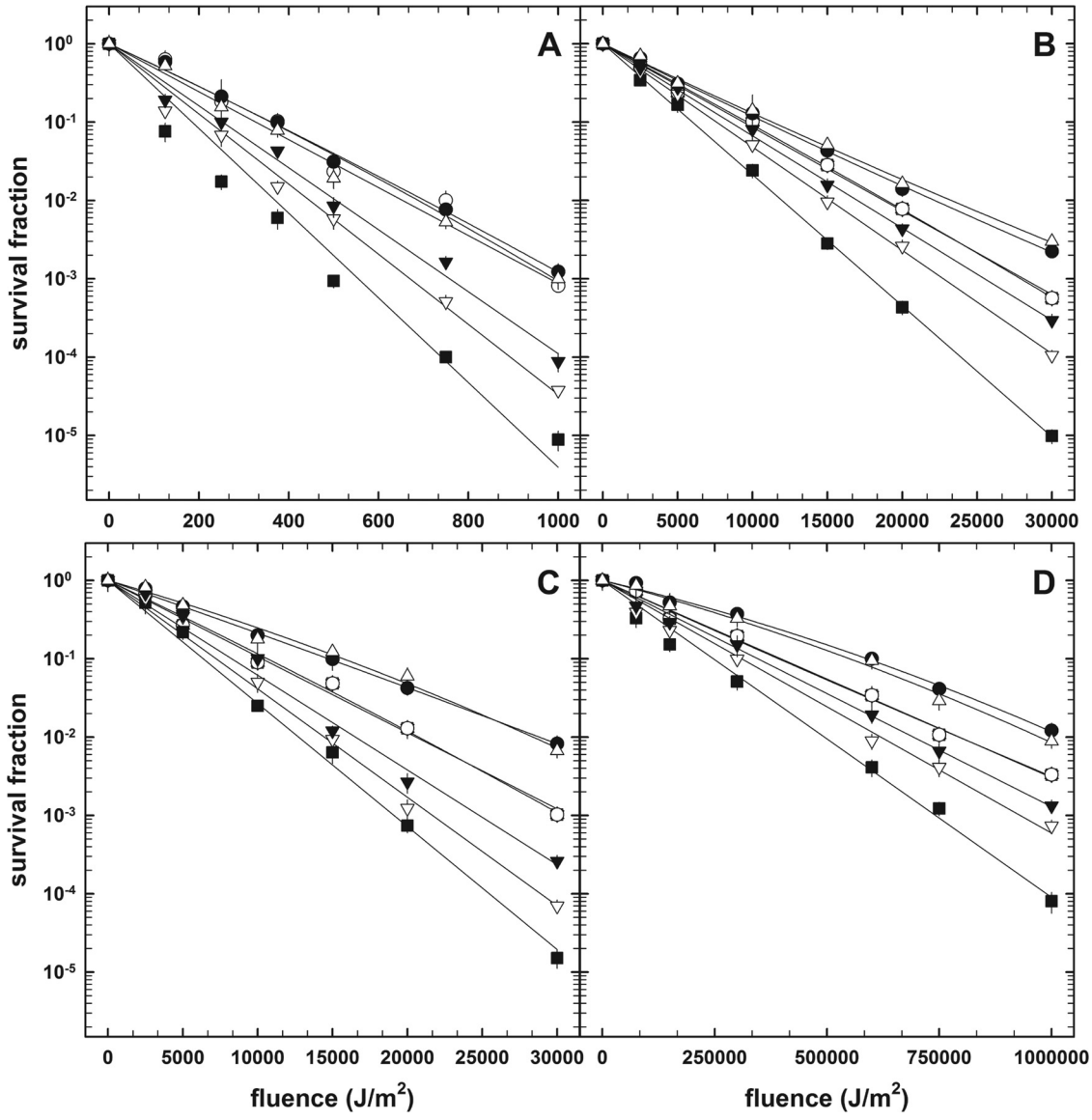

FIG. 1. Survival curves of air-dried spores of *B. subtilis* strains in response to 254-nm UV-C (A), 280- to 400-nm UV-(A+B) (B), 290- to 400-nm UV-(A+B) (C), or 320- to 400-nm UV-A (D) radiation. Strains are indicated as follows: 168 wild type, filled circles; *dacB* mutant, open circles; *sspA* mutant, open downward triangles; *sspB* mutant, filled downward triangles; *sspE* mutant, open upward triangles; and *sspA sspB* mutant, filled squares. Data are expressed as averages and standard deviations ($n = 4$). Error bars for survival data not visible were smaller than the symbol.

TABLE 2. Survival characteristics of air-dried UV-irradiated *B. subtilis* spores[a]

Strain description	F_{10} value with treatment			
	UV-C, 254 nm ($J \cdot m^{-2}$)	UV-(A+B), 280–400 nm ($kJ \cdot m^{-2}$)	UV-(A+B), 290–400 nm ($kJ \cdot m^{-2}$)	UV-A, 320–400 nm ($kJ \cdot m^{-2}$)
Wild type	326 ± 33	10.7 ± 0.9	14.9 ± 0.9	544 ± 50
dacB	295 ± 34	9.0 ± 0.7	10.2 ± 0.8*	377 ± 31*
sspA	169 ± 17*	7.1 ± 0.6*	7.7 ± 0.6*	260 ± 22*
sspB	216 ± 20*	7.9 ± 0.5*	8.4 ± 0.7*	320 ± 23*
sspE	309 ± 28	10.4 ± 0.8	14.7 ± 0.9	513 ± 44
sspA sspB	85 ± 13*	5.8 ± 0.4*	6.9 ± 0.5*	203 ± 16*

[a] Data are averages and standard deviations ($n = 4$). Asterisks indicate survival values that were significantly different from those for wild-type spores ($P \leq 0.05$).

Air-dried spores exposed to UV in the 280- to 400-nm range exhibited exponential inactivation kinetics (Fig. 1B), from which F_{10} values were derived and compared statistically ($n = 4$). The F_{10} values of wild-type and *sspE* and *dacB* mutant spores were not statistically different (Table 2). In contrast, air-dried spores of strains lacking SASP-α, SASP-β, or both were significantly more sensitive to 280- to 400-nm UV than wild-type spores (Table 2).

Air-dried spores exposed to 290- to 400-nm UV, which represents the range of solar UV wavelengths impinging on Earth's surface (33, 34), also exhibited exponential inactivation kinetics (Fig. 1C). The F_{10} values of air-dried wild-type and *sspE* mutant spores were not significantly different at the $P = 0.05$ level (Table 2), and again, spores of strains lacking SASP-α, SASP-β, or both were significantly more sensitive to

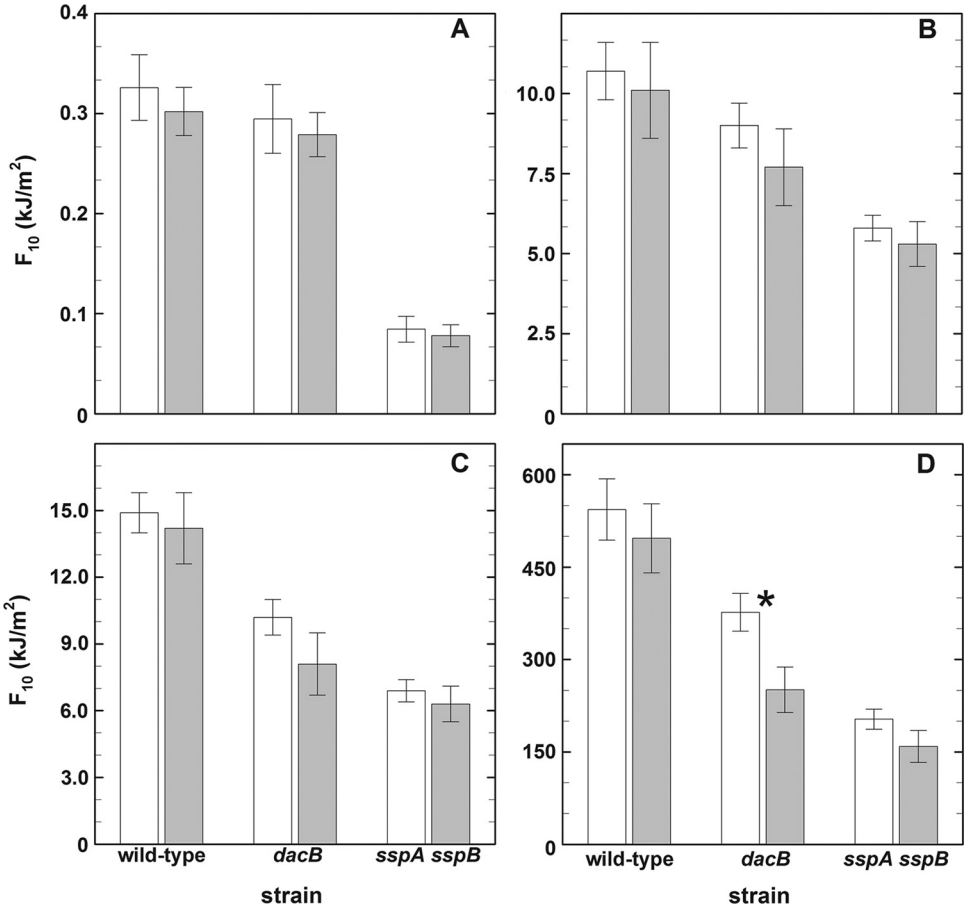

FIG. 2. Comparison of UV resistance (F_{10} values) of spores irradiated either in the air-dried state (white bars) or in aqueous suspension (gray bars) to 254-nm UV (A), 280- to 400-nm UV-(A+B) (B), 290- to 400-nm UV-(A+B) (C), or 320- to 400-nm UV-A (D). Data are averages and standard deviations ($n = 4$ for air-dried spores; $n = 3$ for spores in aqueous suspension). The asterisk denotes a difference significant at a P value of <0.05.

290- to 400-nm UV than wild-type spores (Table 2). Interestingly, air-dried spores of the *dacB* mutant were also significantly more sensitive to UV in the 290- to 400-nm range than wild-type spores (Fig. 1C and Table 2), thus indicating that spore core dehydration (see below) plays a role in spore resistance to environmentally relevant UV wavelengths.

The major part of natural solar UV consists of UV-A, comprising wavelengths of 320 to 400 nm (34, 55), so air-dried spores were exposed to UV-A wavelengths of 320 to 400 nm produced by a solar simulator and an optical filter combination. Spores exposed to 320- to 400-nm UV-A also exhibited exponential inactivation kinetics (Fig. 1D). Comparison of F_{10} values derived from best-fit plots showed the air-dried *sspE* spores lacking SASP-γ were not significantly more sensitive than wild-type spores (Table 2). However, air-dried spores of α/β-type SASP-deficient strains carrying mutations in *sspA*, *sspB*, or *sspA* and *sspB* were significantly more sensitive than wild-type spores to UV-A solar radiation, as were *dacB* spores (Table 2). Thus, α/β-type SASP and decreased spore core water content are also determinants of resistance of air-dried spores to environmental UV-A wavelengths.

Comparison of spore UV resistance in aqueous suspension versus the air-dried state. A previous study showed that spores lacking dipicolinic acid exhibited dramatically different responses to 254-nm UV depending on whether the spores were irradiated in aqueous suspension or in the air-dried state (52). The available data from the present study concerning the UV resistance of wild-type, *dacB*, and *sspA sspB* spores also allowed direct comparisons to be performed on spores exposed under identical conditions of UV irradiation but in either the aqueous (Table 1) or air-dried (Table 2) state. In response to all UV wavelengths tested, spores irradiated in aqueous suspension were slightly more UV sensitive than the same spores irradiated in the air-dried state (Fig. 2); however, in the majority of cases the differences were not significant at the $P < 0.05$ level. Interestingly, the sole exception was that *dacB* spores treated with 320- to 400-nm UV-A radiation were significantly more sensitive in the aqueous state than in the air-dried state ($P = 0.011$) (Fig. 2D).

Increased water content in air-dried *dacB* spores of *B. subtilis*. Previous studies had concluded that *dacB* spores exhibited decreased wet-heat and hydrogen peroxide resistance and contained higher core water content than wild-type spores (40). The observation that *dacB* spores were more sensitive than wild-type spores preferentially to long-wavelength UV (Fig. 1B to D), which is known to produce reactive oxygen species (ROS) especially in the presence of water, led us to hypothesize that even air-dried *dacB* spores may contain more water than air-dried wild-type spores. This notion was tested by de-

TABLE 3. Percentages of water in air-dried spores of PS832 (wild type) and PS1899 (*dacB*)

Strain description	Sample	No. of spores weighed[a]	Mass dried at 20°C, 33% RH (g)	Mass dried at 110°C (g)	% Water	Avg ± SD
Wild type	1	7.62×10^9	1.7×10^{-3}	1.0×10^{-3}	41.2	40.4 ± 2.3
	2	6.73×10^9	1.9×10^{-3}	1.1×10^{-3}	42.1	
	3	9.83×10^9	3.7×10^{-3}	2.3×10^{-3}	37.8	
dacB	1	3.29×10^9	2.3×10^{-3}	8.0×10^{-4}	65.2	64.5 ± 5.5
	2	3.84×10^9	2.3×10^{-3}	7.0×10^{-4}	69.6	
	3	5.67×10^9	4.6×10^{-3}	1.9×10^{-3}	58.7	

[a] Obtained by direct counting.

termining the percentages of water in wild-type and *dacB* spores that had first been air-dried at 20°C with 33% relative humidity (Table 3). The data showed that wild-type spores contained 40.4% ± 2.3% water, a value in good agreement with a range of earlier measurements (32 to 55% water) conducted on spores of *B. subtilis* (10, 48). In contrast, air-dried *dacB* mutant spores contained 64.5% ± 5.5% water, a difference from wild-type spores that was highly significant by Student's *t* test ($P = 0.0086$). Therefore, air-dried *dacB* mutant spores contain significantly more water than do wild-type spores, and it seems highly likely that most of this difference in water content resides in the spore core (see Discussion).

Comparison of UV resistance of spores by inactivation constants. A summary of spore resistance to germicidal 254-nm and environmentally relevant UV radiation for spores of the various strains is presented in Table 4. The IC data showed that killing of spores by simulated natural sunlight was much less efficient and required larger exposure fluences than killing by monochromatic 254-nm UV-C. The data also showed that spore resistance to environmentally relevant UV radiation decreased in the order of wild type (greatest resistance), followed by the *sspE* mutant, the *sspB* mutant, and then the *sspA* mutant, with the *sspA sspB* mutant showing (least resistance), similar to the trend observed after 254-nm UV-C irradiation (Table 4). From these data it can be concluded that SASP-α and -β are important determinants of spore resistance to environmentally relevant UV wavelengths, since spores of mutants lacking SASP-α, SASP-β, or both were significantly more sensitive than wild-type spores at all UV wavelengths tested (Table 4). These results confirm and extend earlier observations that the major α/β-type SASP are important determinants of spore resistance to monochromatic 254-nm UV-C (20, 21). In contrast, spores deficient in SASP-γ did not show any differences in their overall UV resistance from that of wild-type spores at any UV wavelength tested (Table 4), also confirming and extending previous results indicating that SASP-γ plays no role in spore resistance to 254-nm UV-C (12).

In the same manner as that described above, a comparison was made between the IC values of wild-type and *dacB* spores (Table 4). As observed previously (40), *dacB* spores did not differ significantly from wild-type spores in their sensitivity to 254-nm UV-C in a comparison of either their IC (Table 4) or F_{10} values (Table 1). However, *dacB* spores were significantly more sensitive than wild-type spores to 280- to 400-nm UV-(A+B), 290- to 400-nm UV-(A+B), and 320- to 400-nm UV-A (Table 4).

DISCUSSION

Solar UV radiation plays an important role in regulating levels of microorganisms in the environment, and recent decreases in atmospheric ozone levels pose a serious threat to the ecological balance of bacterial populations in the environment (13, 50). In order to survive, bacterial spores must maintain the integrity of their DNA for extended periods (3, 11, 33, 38). Although spores are more resistant to UV radiation than vegetative cells, vegetative cells can constantly repair their DNA. In contrast, spores are metabolically inactive and accumulate DNA damage in their genomes during dormancy (22, 27, 30, 33, 34, 45, 48, 49). Therefore, upon germination, spores must rapidly repair the cumulative damage in their genomic DNA before transcription and replication can resume (33, 34, 38,). While spore DNA photochemistry and DNA repair have been well defined in the laboratory (33–35, 48), the present study of the roles of SASP and spore core water content in spore resistance to natural solar UV radiation provides a better understanding of the resistance of spores in the environment.

The data presented in this article augment previous data

TABLE 4. UV IC[a] of wild-type and SASP- and DacB-deficient *B. subtilis* spores[b]

Strain description	IC (m^2/J) for treatment			
	UV-C, 254 nm	UV-(A+B), 280–400 nm	UV-(A+B), 290–400 nm	UV-A, 320–400 nm
Wild type	$(6.9 \pm 0.7) \times 10^{-3}$	$(1.9 \pm 0.3) \times 10^{-4}$	$(1.6 \pm 0.2) \times 10^{-4}$	$(4.5 \pm 0.4) \times 10^{-6}$
dacB	$(7.0 \pm 0.9) \times 10^{-3}$	$(2.5 \pm 0.4) \times 10^{-4}$	$(2.4 \pm 0.3) \times 10^{-4}$*	$(6.4 \pm 0.5) \times 10^{-6}$*
sspA	$(9.8 \pm 0.8) \times 10^{-3}$*	$(3.1 \pm 0.3) \times 10^{-4}$*	$(3.5 \pm 0.2) \times 10^{-4}$*	$(7.6 \pm 0.8) \times 10^{-6}$*
sspB	$(8.9 \pm 0.6) \times 10^{-3}$*	$(2.8 \pm 0.5) \times 10^{-4}$*	$(3.0 \pm 0.3) \times 10^{-4}$*	$(6.9 \pm 0.7) \times 10^{-6}$*
sspE	$(7.2 \pm 0.7) \times 10^{-3}$	$(2.0 \pm 0.4) \times 10^{-4}$	$(1.6 \pm 0.1) \times 10^{-4}$	$(4.8 \pm 0.5) \times 10^{-6}$
sspA sspB	$(1.1 \pm 0.2) \times 10^{-2}$*	$(4.1 \pm 0.7) \times 10^{-4}$*	$(4.3 \pm 0.8) \times 10^{-4}$*	$(9.6 \pm 1.3) \times 10^{-6}$*

[a] The IC, i.e., the slope of the semilogarithmic survival curves (see Materials and Methods), was determined for each strain and each treatment.
[b] Data are averages and standard deviations ($n = 4$). Asterisks indicate IC values that were significantly different from the respective wild-type IC ($P \leq 0.05$).

suggesting a number of conclusions about mechanisms of spore resistance to monochromatic (254-nm) and environmentally relevant polychromatic UV radiation at >290 nm. First, it appears that the major α/β-type SASP are necessary not only for spore UV-C resistance in the laboratory (20) but also for spore resistance in the solar UV environment (this report). In contrast, SASP-γ does not appear to be involved in spore resistance either to laboratory UV-C (12) or to environmental UV (this report). An unexpected result from this work was the observation that spores of strains carrying a *dacB* mutation with higher spore core water content than wild-type spores were not significantly more sensitive than wild-type spores to monochromatic 254-nm UV-C (40; this report) but were significantly more sensitive to environmentally relevant solar UV-B and UV-A wavelengths, particularly to UV wavelengths of >320 nm (this report).

A body of evidence has accumulated which suggests that solar UV exerts its lethal effects through direct interaction with spore DNA and indirectly via the generation of ROS, such as peroxide, superoxide, or hydroxyl radicals (34, 55). Examples for direct UV damage include pyrimidine dimers, such as the "spore photoproduct" (SP) 5-thyminyl-5,6-dihydrothymine, cyclobutane pyrimidine dimer, and pyrimidine(6-4)pyrimidone photoproducts (5, 53). These direct photoproducts are produced much more efficiently by 254-nm UV-C and the shorter-wavelength UV-B (290 to 320 nm) component of sunlight than they are by longer-wavelength UV-A (320 to 400 nm) (54). In contrast, nonspecific DNA damage, such as DSB, single-strand breaks, and AP sites, are more likely to be produced indirectly from UV-A via the production of ROS (51, 55, 59). It is well established that SP is the major UV photoproduct in spore DNA irradiated with UV-C (4, 56). While SP is also the major photoproduct in DNA of spores exposed to sunlight, less SP is detected per lethal event at solar wavelengths, suggesting that additional DNA photoproducts are also formed in spores exposed to solar radiation (36, 51, 54, 56, 59). Slieman and Nicholson (51) reported that cyclobutane pyrimidine dimers are preferentially produced in spores exposed to the UV-B component of sunlight and that DSB and single-strand breaks are formed mainly when spores are exposed to the UV-A component of sunlight (27, 51). However, AP sites were not detected in spore DNA under any of the conditions tested (51).

Regarding spore resistance to oxidative stress, it has been well established that UV-B radiation and UV-A radiation are both capable of inducing cellular oxidative stress via production of ROS and by photolysis of H_2O water molecules into H· and OH· radicals (2, 13, 55). It has been observed that spores deficient in their α/β-type SASP but not SASP-γ are much more sensitive to treatments which produce ROS (e.g., ionizing radiation or oxidizing agents, such as hydrogen peroxide) than are wild-type spores (16, 24, 26, 33, 34, 48). Likewise, Popham et al. (40) reported increased hydrogen peroxide sensitivity of spores carrying the *dacB* mutation, suggesting that increased spore core water content results in decreased spore resistance to oxidative damage. In this study, we observed that *dacB* spores were also significantly more sensitive to solar wavelengths of UV-B and UV-A than wild-type spores, while *dacB* spores exhibited the same resistance as wild-type spores to 254-nm UV-C.

Mutation in *dacB* leads to higher core water content in wet spores, as determined by buoyant density centrifugation (40), but little was known about the water content of wild-type and *dacB* spores in the air-dried state. We determined that air-dried *dacB* spores also contained considerably larger amounts of water than did wild-type spores (Table 3). We reason that the extra water in *dacB* spores also likely resides in the spore core, since the outer spore layers (the spore coat and the cortex) are readily permeated by water and have been observed to shrink and swell dynamically with changes in relative humidity (7, 39, 58). It is thought that the cortex constrains expansion and contraction of the spore core, and the inner spore membrane also acts to greatly slow water passage, keeping the (de)hydration state of the spore core constant (7). These observations lead us to the conclusion that in addition to the α/β-type SASP, spore core dehydration is an important factor in the resistance of spores to environmentally relevant solar UV radiation, particularly the longer UV-A wavelengths, and that core dehydration specifically protects spore DNA by virtue of reducing the production and/or migration of ROS within the spore core.

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