APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1988, p. 1275–1276
0099-2240/88/051275-02$02.00/0

Vol. 54, No. 5

NOTES

Decreased UV Light Resistance of Spores of *Bacillus subtilis* Strains Deficient in Pyrimidine Dimer Repair and Small, Acid-Soluble Spore Proteins

BARBARA SETLOW AND PETER SETLOW*

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

Received 30 November 1987/Accepted 3 February 1988

Loss of small, acid-soluble spore protein α reduced spore UV resistance 30- to 50-fold in *Bacillus subtilis* strains deficient in pyrimidine dimer repair, but gave only a 5- to 8-fold reduction in UV resistance in repair-proficient strains. However, both repair-proficient and -deficient spores lacking this protein had identical heat and γ-radiation resistance.

Vegetative cells of *Bacillus subtilis* are 5- to 10-fold more sensitive to killing by UV light than are dormant spores (5). This difference in UV resistance is due to the formation of pyrimidine dimers in vegetative cell DNA but not spore DNA upon UV irradiation (2, 12). In contrast, a thyminyl-thymine adduct (originally termed the spore photoproduct) is the major photoproduct formed in spore DNA after UV irradiation. There are at least two mechanisms for efficiently eliminating this spore photoproduct, including one which is specific for spore photoproduct repair under control of the *ssp* locus (2, 10, 11). As would be predicted from these results, strains which are deficient in excision repair of pyrimidine dimers show increased UV sensitivity as vegetative cells, although their spores show relatively normal levels of UV resistance (10, 11).

While our understanding of the causes of the difference in UV photochemistry between spore and cell DNA in vivo is not complete, a key role appears to be played by the large amount of small, acid-soluble spore protein (SASP) present in bacterial spores (3). Thus, *B. subtilis* spores lacking one or two of the major SASPs, termed SASP-α and -β, are 8- to 10-fold more UV sensitive than are wild-type spores (8, 9), and pyrimidine dimers are formed in DNA after UV irradiation of spores which lack these major SASPs (12).

The observation that UV irradiation of spores lacking major SASPs results in pyrimidine dimer formation leads to the prediction that the UV resistance of such spores should be decreased in strains which are deficient in excision repair of pyrimidine dimers. Consequently, we deleted the genes for one or two major SASPs from several strains with mutations in UV repair genes and measured the UV resistance of the spores of the resulting strains, as well as their heat and γ-radiation resistance. The *B. subtilis* strains used were 168 (D. Tipper, University of Massachusetts Medical School, Worcester), GSY1026 and FB56 (R. Slepecky, Syracuse University), and 1A345, 1A488, and 1A489 (Bacillus Genetic Stock Center). For most strains (Table 1), we deleted the gene which codes for SASP-α, since this protein is the most predominant SASP of the class which plays a role in spore UV resistance (1, 9). Previous work has shown that loss of SASP-α alone results in UV-sensitive spores (8, 9). Strains were made competent and transformed to chloramphenicol resistance with an integratable plasmid carrying the gene coding for SASP-α, which contained a deletion removing the gene's promoter, ribosome-binding site, and amino-terminal coding region (1). Drug-resistant transformants were grown in liquid culture, and chromosomal DNA was isolated. The chromosomal DNA was screened by restriction enzyme digestion and Southern blotting to identify strains carrying two copies of the gene carrying the deletion (1, 8). These strains are designated -α^-. Strain 1A489α^- was further engineered to lose the chloramphenicol resistance marker and then transformed with a second plasmid carrying the gene coding for SASP-β which contained a deletion similar to that described above (1). Drug-resistant transformants were screened as described above (1, 8). This created an $\alpha^-\beta^-$ strain. All strains were sporulated in 2× SG medium (4) with thymine (50 μg/ml) if necessary; spores were cleaned and stored as described previously (1). All spore preparations used were >98% free of vegetative cells or germinated spores, and appropriate SASPs were absent from spores of α^- or $\alpha^-\beta^-$ strains (data not shown) (1, 4).

As found previously, spores of α^- strains which are proficient in excision repair of pyrimidine dimers (strains 168, 1A488, and GSY1026), were 5 to 8.5 times more UV sensitive than spores of the corresponding α^+ strains (8) (Table 1). In contrast, spores of α^- strains which are deficient in excision repair (strains 1A345 and FB56), were 30 to 50 times more UV sensitive than the corresponding α^+ spores (Table 1). However, in strain 1A489, which is deficient in both excision repair and spore photoproduct repair, loss of SASP-α had only a small effect on UV resistance; the UV sensitivity of spores of this strain was not increased by conversion to an $\alpha^-\beta^-$ strain (Table 1). Strikingly, α^- spores of all strains analyzed had similar levels of heat resistance, as did all α^+ spores. However, as found previously, α^- spores were more heat sensitive than α^+ spores (Table 1). Strikingly, the most UV-sensitive spores had a level of γ-radiation resistance identical to that of wild-type spores (Table 1).

The results presented in this communication allow three distinct conclusions. First, as noted previously, loss of

* Corresponding author.

TABLE 1. Resistance of spores of various strains to heat, UV, and γ radiation

B. subtilis strain	Genotype[a]	Resistance[b]		
		UV radiation	Heat	γ radiation
168	*trpC2*	3,420	85	180
168 α^-	*trpC2* α^-	395	11	175
GSY1026	*trpC2 metB4*	2,870	—	—
GSY1026 α^-	*trpC2 metB4* α^-	568	—	—
FB56	*trpC2 metB4 uvrA42*	4,310	—	—
FB56 α^-	*trpC2 metB4 uvrA42* α^-	141	—	—
1A345	*met-14 thyA1 thyB1 sul trpC2 uvrA42*	2,180	78	
1A345 α^-	*met-14 thyA1 thyB1 sul trpC2 uvrA42* α^-	44	14	—
1A488	*met-14 thyA1 thyB1 sul trpC2 ssp-1*	1,100	80	—
1A488 α^-	*met-14 thyA1 thyB1 sul trpC2 ssp-1* α^-	222	14	—
1A489	*met-14 thyA1 thyB1 sul trpC2 ssp-1 uvrA42*	69	85	185
1A489 α^-	*met-14 thyA1 thyB1 sul trpC2 ssp-1 uvrA42* α^-	19	13	—
1A489 $\alpha^-\beta^-$	*met-14 thyA1 thyB1 sul trpC2 ssp-1 uvrA42* $\alpha^-\beta^-$	21	15	195

[a] The nomenclature of the *ssp* locus, in which a mutation (*ssp-1*) gives rise to UV-sensitive spores, is somewhat confusing. While it was initially termed the *ssp* locus (10, 11), this gene has never been mapped. Consequently, the mnemonic *ssp* is now used to describe SASP genes (1). However, in this communication we use the original nomenclature.

[b] All killing curves were semilogarithmic over at least 2 logs of killing. Values presented are averages of at least two independent determinations. Values are shown as follows: UV radiation, ergs per square millimeter required to kill 90% of the population; heat, minutes of incubation at 87.5°C required to kill 90% of the population; γ radiation, kilorads required to kill 90% of the population. —, Not tested.

SASP-α or SASP-α and -β has no effect on spore γ-radiation resistance, nor does the absence of wild-type *uvrA* or *ssp* genes (6, 10). Second, the absence of wild-type *uvrA* and *ssp* genes does not affect spore heat resistance, since we found that spores of all α^+ strains had similar heat resistance, as did spores of all α^- strains. Previous work has indicated that spores of *recA* strains are slightly more heat sensitive than isogenic *rec*$^+$ spores (7). This does not appear to be the case for spores with lesions in other UV repair genes. The third conclusion, and the major one, is that spores of α^- strains with a mutation in a gene for excision repair of pyrimidine dimers are significantly more UV sensitive than are spores of corresponding excision-repair-proficient α^- strains. Since the α^- mutation results in production of pyrimidine dimers in spore DNA after UV irradiation, this suggests that the production of these dimers is the reason for the UV sensitivity of α^- and $\alpha^-\beta^-$ spores.

This work was supported in part by a grant from the U.S. Army Research Office.

LITERATURE CITED

1. **Connors, M. J., J. M. Mason, and P. Setlow.** 1986. Cloning and nucleotide sequencing of genes for three small, acid-soluble proteins from *Bacillus subtilis* spores. J. Bacteriol. **166**:417–425.
2. **Donnellan, J. E., Jr., and R. B. Setlow.** 1963. Thymine photoproducts but not thymine dimers found in ultraviolet irradiated bacterial spores. Science **149**:308–310.
3. **Fliss, E. R., M. J. Connors, C. A. Loshon, E. Curiel-Quesada, B. Setlow, and P. Setlow.** 1985. Small, acid-soluble spore proteins of *Bacillus*: products of a sporulation-specific, multigene family, p. 60–66. *In* J. A. Hoch and P. Setlow (ed.), Molecular biology of microbial differentiation. American Society for Microbiology, Washington, D.C.
4. **Goldrick, S., and P. Setlow.** 1983. Expression of a *Bacillus megaterium* sporulation-specific gene during sporulation of *Bacillus subtilis*. J. Bacteriol. **155**:1459–1462.
5. **Gould, G. W.** 1983. Mechanisms of resistance and dormancy, p. 173–209. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press, Inc., New York.
6. **Hackett, R. H., and P. Setlow.** 1988. Properties of spores of *Bacillus subtilis* strains which lack the major small, acid-soluble protein. J. Bacteriol. **170**:1403–1404.
7. **Hanlin, J. H., S. J. Lombardi, and R. A. Slepecky.** 1985. Heat and UV light resistance of vegetative cells and spores of *Bacillus subtilis rec* mutants. J. Bacteriol. **163**:774–777.
8. **Mason, J. M., and P. Setlow.** 1986. Essential role of small, acid-soluble spore proteins in resistance of *Bacillus subtilis* spores to UV light. J. Bacteriol. **167**:174–178.
9. **Mason, J. M., and P. Setlow.** 1987. Different small, acid-soluble proteins of the α/β type have interchangeable roles in the heat and UV resistance of *Bacillus subtilis* spores. J. Bacteriol. **169**:3633–3637.
10. **Munakata, N., and Y. Ikeda.** 1968. A mutant of *Bacillus subtilis* producing ultraviolet-sensitive spores. Biochem. Biophys. Res. Commun. **33**:469–475.
11. **Munakata, N., and C. S. Rupert.** 1975. Effects of DNA-polymerase-defective and recombination-deficient mutations on the ultraviolet sensitivity of *Bacillus subtilis* spores. Mutat. Res. **27**:157–169.
12. **Setlow, B., and P. Setlow.** 1987. Thymine-containing dimers as well as spore photoproducts are found in ultraviolet-irradiated *Bacillus subtilis* spores that lack small acid-soluble proteins. Proc. Natl. Acad. Sci. USA **84**:421–424.