A MUTANT OF *BACILLUS SUBTILIS* PRODUCING ULTRAVIOLET-SENSITIVE SPORES

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In general, bacterial spores are more resistant to ultraviolet (UV) irradiation than the vegetative cells of the same strain (Zelle and Hollaender,1955)(Donnellan and Stafford,1968). It is thought that the spores might be protected against UV effects by a special mechanism.

This paper deals with isolation of a mutant of *Bacillus subtilis* producing UV-sensitive spores. This mutant was derived from a mutant producing UV-sensitive vegetative cells. In the new isolate, the spores were almost as sensitive to UV irradiation as the vegetative cells. Spore-specific protection or repair mechanism is discussed.

MATERIALS AND METHODS

Bacterial strains. A thymine and indole requiring mutant (*thy⁻ ind⁻*$_{168}$, kindly supplied by Dr. H. Hirokawa) of *Bacillus subtilis* Marburg was used as a UV-resistant (*uvr*) strain. Mutants producing UV-sensitive vegetative cells (*uvs*) were also used. These mutants were derived from the *uvr* strain and marked with auxotrophic markers by the transformation technique. They are host-cell reactivation negative except one strain (*uvs-80*) which has been demonstrated to be recombination defective. UV-sensitivities of these strains are to be published elsewhere.

Media. Spores were prepared on Schaeffer's sporulation medium (SNB, Takahashi,1965). Vegetative cells were prepared in Penassay broth (Difco-antibiotic medium 3, 1.75g in 1,000ml). Dilution buffer contained, in 1,000ml water, 10ml of tris(hydroxymethyl)-aminomethane buffer (1M, pH 7.2), 10ml of $MgCl_2$ (1M), and 5g NaCl.

Preparation of spores. Cells incubated on SNB agar plates at 37° for 48 hours were collected in a saline-EDTA solution (0.15M NaCl and 0.1M ethylenediaminetetraacetate, pH 8.0) and treated with lysozyme (1mg/ml) at 37° for 60 minutes. After the treatment, survived spores were washed three times with distilled water. The spores suspended in 1ml of distilled water were purified further by the aqueous polymer two-phase system (Sacks and Alderton, 1961) using 0.78ml of 0.3M potassium phosphate buffer (pH 7.3) and 0.5ml of polyethylene glycol 4,000 (50 % w/v solution). The mixture was agitated vigorously and subjected to centrifugation (1,500g for 5 minutes). Purified spores were recovered from the upper phase and washed five times with dilution buffer.

UV-irradiation. Vegetative cells or purified spores were exposed to UV (3.4 ergs/mm^2) in dilution buffer (about 10^6/ml). Survivors were counted on SNB plates.

Gamma-irradiation. Spores in dilution buffer (about 10^8/ml) were exposed to gamma-rays (3,300 R/min) from a Cs^{137} source. Survivors were counted on SNB plates.

RESULTS

UV-sensitivities of uvs spores

Figure 1 shows survival curves of the spores of uvr and uvs-42 strains after exposure to UV. Spores of other uvs strains including uvs-80 exhibited the same order of UV-sensitivities. Figure 2 shows survival curves of the vegetative cells of uvr and uvs-42 strains. From the comparison of the data in Figures 1 and 2, it may be concluded

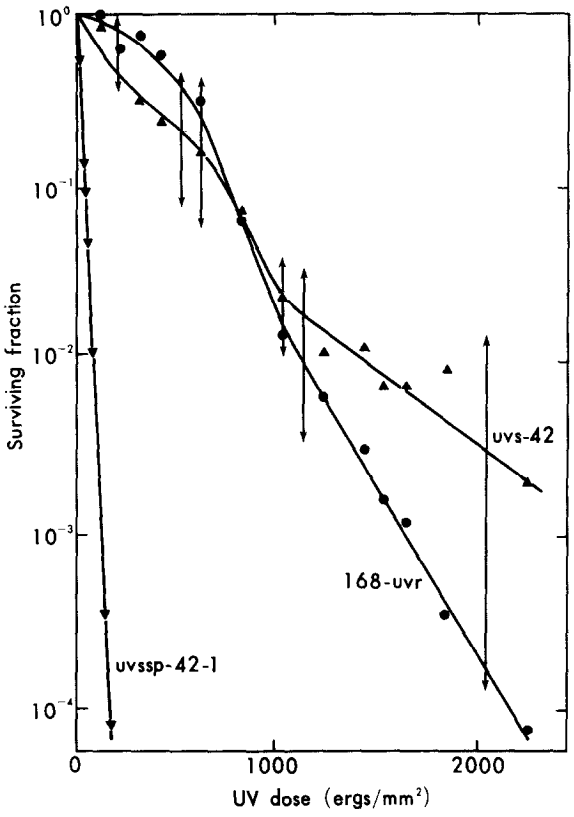

Figure 1. UV-sensitivities of spores of uvr (●), uvs-42 (▲), and uvssp-42-1 (▼). The arrows indicate the deviation widths of the survival fractions of 19 uvs strains.

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that the spores of uvs strains are not so sensitive to UV irradiation as the vegetative cells.

Isolation of a mutant producing UV-sensitive spores

Spores of uvs-42 ($thy^-met_{14}^-ind_{168}^-$ sulfanilamide resistant) were exposed to gamma-rays from a Co^{60} source to a 10^{-4} surviving fraction and survivors were spread on SNB plates. After incubation at 37° for 48 hours, each plate was replica-printed onto two sets of fresh plates. One set of plates were exposed to UV (10^4 ergs/mm^2) and incubated at 37° for 48 hours. The colonies that failed to grow on the irradiated plates were picked up from the un-irradiated plates.

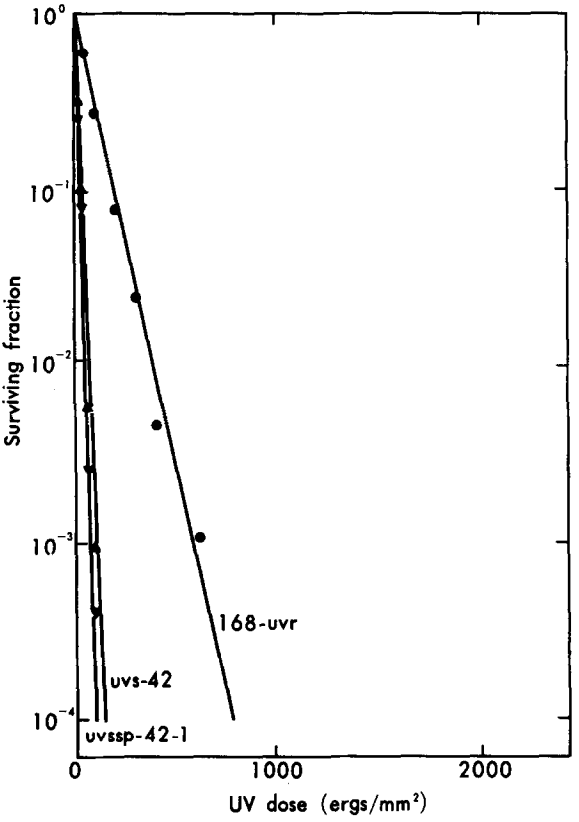

Figure 2. UV-sensitivities of vegetative cells of uvr (●), uvs-42 (▲), and uvssp-42-1 (▼).

The isolates were purified by the single colony isolation technique and, finally, three strains named uvssp-42-1, uvssp-42-2, and uvssp-42-3 were obtained. The yield was lower than 0.1% of the replica-printed colonies. Among the three strains, uvssp-42-1 produced spore-like bodies which could not be regarded as genuine spores. Remaining two strains produced genuine spores, but the spores of uvssp-42-3 germinated at a quite low frequency.

Survival curves of the spores and vegetative cells of uvssp-42-1 are shown in Figures 1 and 2. The data indicate that the spores are about fifteen times more sensitive to UV irradiation than the spores of uvr and uvs strains. The vegetative cells are also

sensitive to UV irradiation, but the degree is of the order of uvs-42.

Characterization of uvssp-42-1 spores

The view that the spores of uvssp-42-1 are genuine spores was drawn from the following observations. (1) The spores were resistant to the treatment with lysozyme (1mg/ml) and sodium lauryl sulphate (1%) in saline-EDTA solution. (2) The spores were stained with malachite green, but not with safranin. (3) The spores could survive the heating at 85° for 15 minutes. (4) The spores contained 6.2 % dipicolinic acid. The content of uvs-42 spores was 7.3 %. The measurements were carried out by the colorimetric method of Janssen et al. (1958). (5) When the spores were inoculated at 37° in minimum medium of Spizizen (1958) supplemented with L-alanine (200μg/ml) and adenine (20μg/ml), the optical density at 530 mμ dropped to

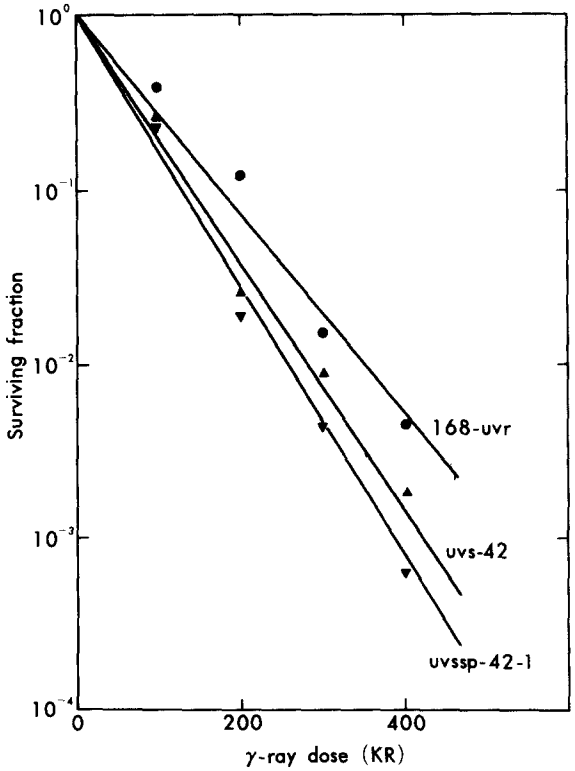

Figure 3. Gamma-ray sensitivities of spores of uvr (●), uvs-42 (▲), and uvssp-42-1 (▼).

50 % of the initial value within 25 minutes. The germinated spores were stained with safranin.

Sensitivity of uvssp-42-1 spores to gamma-rays

Spores of uvr, uvs-42, and uvssp-42-1 were exposed to gamma-rays from a Cs^{137} source. The data in Figure 3 suggest that the spores of uvssp-42-1 are 1.4 fold more sensitive to gamma-rays than the spores of uvr and 1.1 fold more sensitive than the spores of uvs-42. We would not like to think the differences are highly significant.

DISCUSSION

As stated above, (1) the spores of uvs strains are not so sensitive to UV irradiation as the vegetative cells of the same strain, (2) a mutation can make bacterial spores highly sensitive to UV irradiation without giving any significant effect on the UV sensitivity of vegetative cells. These facts suggest that bacterial spores may be protected against UV irradiation by a spore-specific protection or repair mechanism. If the protection mechanism is valid, it may be effective only against UV light since the spores of uvssp-42-1 are not sensitive to gamma-rays. If the repair mechanism works in the spores or germinated spores, it may be different from the dark-repair mechanism in the vegetative cells. The spores of host-cell reactivation negative strains are not always sensitive to UV irradiation.

In the present study, the UV-sensitive spore mutants were derived from a uvs strain. The question whether or not the uvssp mutations are completely independent from the uvs mutation will be answered by isolation of uvssp mutants directly from a uvr strain and also by genetic analysis. Studies along this line are now in progress.

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