SHORT COMMUNICATION

J. Gen. Appl. Microbiol., **16**, 181–184 (1970)

UV-RESISTANCE OF TRANSFORMING DNA IN GERMINATING SPORES OF *BACILLUS SUBTILIS*

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(Received October 20, 1969)

Resistance of bacterial spores to ultraviolet ray (UV) increases temporarily in the course of germination (1–4). STAFFORD and DONNELLAN (3) showed that this increase of UV-resistance in germinating spores was correlated with a decrease in the production of thymine-containing photoproducts. The experiments described below were carried out to see if this increase in UV-resistance could be explained in terms of the resistance of DNA in the spores as measured by transforming ability.

Spores of *Bacillus subtilis* Marburg 160 *try*⁻ *arg*⁻ were prepared as described by TANOOKA (5). To facilitate the extraction of DNA from spores (6), it was necessary to sensitize the spores to lysozyme (7) prior to germination and UV-irradiation. The sensitization to lysozyme was performed by treating the dormant spores (about 10⁹ spores/ml) at 37° for 90 min with 10% thioglycolic acid in the presence of 7.2 M urea and 0.5 mM dithiothreitol (8). The spores thus treated were centrifuged, washed twice with 0.1 M phosphate buffer (pH 7.8), and suspended in distilled water. At this stage, the spores were highly dormant and required heat-shock for germination.

After being heat-shocked at 70° for 2 hr, the spores (about 10⁹ spores/ml) were germinated at 37° in 10 mM Tris buffer (pH 7.0) containing 10 mM L-alanine. As shown in Fig. 1, these spores exhibited an unusually long lag period (30 min or more at this spore concentration) before commencement of germination as measured by optical density. This lag period tended to increase with spore concentration. Despite these abnormal behaviors caused by the thioglycolic acid treatment, germination of these spores was considered to be physiological in nature because (1) they were fully viable and (2) no appreciable change in optical density occurred in the absence of alanine.

For the examination of UV-resistance of DNA in spores, an aliquot (5 ml) removed from the germinating spore suspension was chilled quickly to below 10°, transferred to an aluminium dish (76 mm in diameter) and sub-

Fig. 1. Changes in UV-resistance of transforming DNA in thioglycolic acid-treated spores during germination.

○ Optical density of the spore suspension at 600 m μ (relative to time zero).

△ Survival of transforming ability of DNA in spores after UV-irradiation for 2 min.

Germination curve (decrease in optical density) for non-treated spores (heat-shocked at 70° for 2 hr) is inserted for comparison (□).

jected to UV-irradiation with shaking. A 15-W germicidal lamp (Mazda) was used as the UV-source, placed at a definite distance (20 cm) from the sample.

After irradiation, the spores were centrifuged and treated with lysozyme (0.1 mg/ml) at 37° for 30 min in hypertonic solution (0.5 ml) containing 25% lactose, 0.01 M Tris buffer (pH 8.0), 0.01 M EDTA, and 0.15 M NaCl (6). Procedures for the extraction of DNA from the lysozyme-treated spores by osmotic shock and for the assay of its transforming ability were virtually identical with those described by TANOOKA and SAKAKIBARA (6). A mutant of Marburg strain deficient of dark-repair (168 *uvr-42 thy⁻ met₁₄⁻ hcr⁻*) isolated by MUNAKATA and IKEDA (9) was used as the recipient and the transformants to *met⁺* were counted (5).

The change in UV-resistance of transforming ability of DNA in spores during germination is shown in Fig. 1. In this experiment, all the samples were irradiated for a fixed period (2 min). It is clear that DNA in spores becomes highly resistant when the spores are actively germinating. This fact corresponds to the change of UV-resistance in spores, as measured by colony-forming ability, during germination (2-4). Survival of transforming ability of DNA in non-germinating spores, germinating spores, and vegeta-

Fig. 2. Survival curves for transforming ability of DNA.

- non-germinating spores (heat-shocked).
- non-germinating spores (non-heat-shocked).
- △ germinating spores (at 53 min of germination).
- vegetative cells.

The vegetative cells from the late exponential growth phase were irradiated in saline and DNA was extracted as in the case of spores.

tive cells as a function of UV-exposure is shown in Fig. 2. A remarkable upward bending of the survival curve for DNA in germinating spores suggests the appearance of a highly UV-resistant fraction in the total population.

These results, together with the photochemical evidence obtained by STAFFORD and DONNELLAN (3), indicate that the extreme UV-resistance of spores at the transient stage in germination (4) is due to the specific state of intracellular DNA at this stage.

We are indebted to Dr. H. Tanooka, National Cancer Center Research Institute, for his valuable advice and a gift of the bacterial cultures.

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