Relationship of Dipicolinic Acid Content in Spores of Bacillus cereus T to Ultraviolet and Gamma Radiation Resistance

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Spores of Bacillus cereus T lacking dipicolinic acid showed a statistically significant reduction in resistance to ultraviolet and γ radiation as compared with spores with high dipicolinic acid content.

The high content of dipicolinic acid (DPA) in bacterial spores has been variously linked with their exceptional hardness to physical and chemical stresses. The heat resistance of Bacillus cereus T was shown to vary directly with DPA content (4), and the appearance of heat resistance during sporulation coincided with DPA synthesis (6, 17).

However, for radiation resistance no such correlation has been established. Resistance to radiation appeared during forespore development, some 2 hr prior to DPA synthesis (12, 15, 16), although a small secondary increase continued throughout the later stages of spore development and was possibly associated with DPA synthesis and Ca uptake (17). On the other hand, spores with low DPA content have the same or even greater radiation resistance than normal spores (2, 18). Thus, the question of the relation of DPA to radiation resistance remains controversial.

In the present experiments spores of B. cereus T, DPA+ and DPA− (19), have been compared in an attempt to clarify the role of DPA in radiation resistance.

The spores were prepared as described by Halvorsen and Swanson (5) except that modified G medium was used (9) and the harvested spores were stored in distilled water at 4°C. DPA− spores contained no detectable DPA by the colorimetric assay of Rothman and Fields (11), whereas DPA+ spores contained 10.2%. For irradiation, samples were standardized at 10⁶ spores/ml in distilled water.

Ultraviolet (UV) irradiation was performed at room temperature with a General Electric germicidal lamp (253.7 nm) at a dose rate of 24 ergs/mm²/sec. Samples of 2 ml were irradiated in uncovered glass petri plates while they were magnetically stirred. To prevent possible photoreactivation, irradiated cells were handled in low intensity yellow light.

Gamma irradiation of aqueous anoxic spore suspensions of 1.5 ml was performed in wet ice in a Cs-137 Gammator (Model 34-3, Radiation Machinery Corp., Parsippany, N.J.) at a dose rate of 0.1 Mrad/hr.

Survivors were evaluated by colony counts on plates of modified G medium (9) supplemented with 2% agar (19). Incubation was at 30°C for 24 hr.

Figures 1 and 2 show the UV and gamma radiation survival curves of B. cereus T DPA− as compared to DPA+. Three to six replicate samples were used for each radiation dose, with three plates per replicate; standard errors are indicated by vertical bars. The curves in Fig. 1 and 2 were obtained by a least square fit (13) of the survival points. A significance test on the slopes of the four lines showed that the data fit a straight line (P = 0.05), except for DPA− UV resistance, which did not fit a straight line at this significance level. The UV survival curves (Fig. 1) exhibited multitarget inactivation kinetics with an apparent shoulder up to 200 ergs/mm². The gamma-survival curves (Fig. 2) were of a diphasic nature, indicating perhaps that the spore populations were heterogeneous, that radiation effects may not have been uniform during the course of gamma exposure, or that spores may become adapted to gamma rays during irradiation in the same sense that spores become adapted to heat during the course of thermal inactivation (1). An analysis of variance (13) revealed a significant

1 The data in this paper are taken from material to be presented by P. E. Berg in partial fulfillment of the requirements for the Ph.D. degree at the Illinois Institute of Technology, Chicago.
The difference between DPA\(^+\) and DPA\(^-\) (\(P = 0.05\)) for both UV and gamma radiation, i.e., in both cases the DPA\(^-\) mutant exhibited a statistically significant reduction in radiation resistance.

It may be postulated that DPA could protect by (i) specific absorption of photons in the UV range, where DPA absorbs strongly; (ii) creation of an essentially dry spore interior (7) by deposition of DPA-chelates (10, 14); and (iii) stabilization of spore biopolymers by coordinative binding involving DPA and probably divalent cations (8, 14). Mechanism (i) may be especially important in UV protection since DPA is thought to concentrate in the cortex (8), where it may conceivably form a protective layer for the interior regions of the spore which contain the DNA. Protection by DPA against gamma rays by mechanism (ii) may have reduced the yield of water radicals responsible for indirect action. Mechanism (iii) is postulated for gamma protection since DPA was demonstrated to reduce inactivation of enzymes by ionizing particles in model systems (3).

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LITERATURE CITED