CHARACTERISTICS OF AN ULTRAVIOLET IRRADIATION SENSITIVE STRAIN OF BACILLUS SUBTILIS

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A transformable, ultraviolet (UV) sensitive strain of Bacillus subtilis was isolated to examine the dark repair (DR) system in this species, and its relationship to the mechanism of transformation with irradiated DNA.

Experimental findings indicate that B. subtilis is capable of DR but, contrary to expectation, there is little if any repair of intracellular transforming DNA. Since radiation resistant B. subtilis cells do not show any increased UV sensitivity during their period of competence, the physical state of the intracellular transforming DNA may account for the absence of DR.

Materials and Methods. Bacterial Strains -- Strain 168UV$^-$ was transformed to tryptophan deficiency with DNA prepared from a strain 168UV$^+$ T$^-$. Strain SB-1A$^-$ is deficient for histidine$_1$, tryptophan$_2$ and arginine, and strain SB-25 for histidine$_2$ and tryptophan$_2$.

DNA and Transformation -- Bacterial DNA was prepared by the method of Marmur (1961). The preparation of competent cells and transformation procedures have been described previously, (Mahler et al., 1963).

Phages -- Phages SP3, isolated by Romig (1962), and SP82, isolated by Green (1964), were plated on the special media suggested by these authors. Acriflavin (Nutritional Biochemicals Corporation) when required was added to the top and bottom agar at a concentration of 0.5 ug/ml.

X and UV Irradiation -- A 100 kv Philips source with a 0.78 mm aluminum

filter was used for X irradiation. Bacteriophages and bacteria were irradiated in broth to protect against indirect X ray effects. A 15-watt germicidal lamp (G.E.) was used as a source of UV. Irradiation was carried out in a black box with the sample 45 cm from the lamp.

Results -- A UV sensitive, transformable mutant of B. subtilis 168, 168UV$^-$, was isolated by the method of Hill (1958). Although very susceptible to irradiation at λ2573Å (Fig. 1), strain 168UV$^-$ shows no enhanced sensitivity to X-irradiation, unlike the radiation-sensitive strains of Escherichia coli K12 AB1886

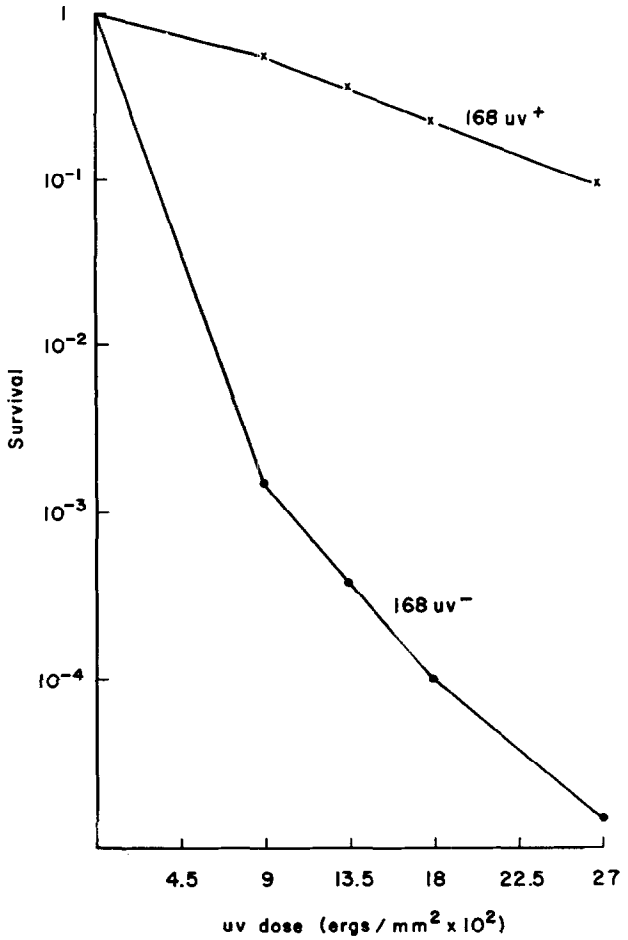

Fig. 1. Ultraviolet survival curves for B. subtilis strains 168UV$^+$ and 168UV$^-$. Cells grown to a concentration of approximately 1 x 10^8/ml in minimal salts solution, supplemented with 0.5% glucose and 0.1% casein hydrolysate, were irradiated and plated on nutrient agar.

(Howard-Flanders et al., 1962) and B_{s-1} (Hill, 1958).

Boyce and Howard-Flanders (1964) and Setlow and Carrier (1964) have shown that the UV sensitivity of these E. coli strains is caused by a lack or defect of DR and Metzger (1964) has suggested that the phenomenon of host cell reactivation (HCR) of irradiated phage is based on the same mechanism as DR. HCR by strain $168UV^-$ therefore was tested to determine the basis of its UV sensitivity.

Phage Experiments -- B. subtilis $168UV^+$ and $168UV^-$ were infected with UV irradiated phage SP3. Fig. 2 shows the decreased survival of the irradiated phage when plated on the UV sensitive host. Inhibition of HCR could also be ob-

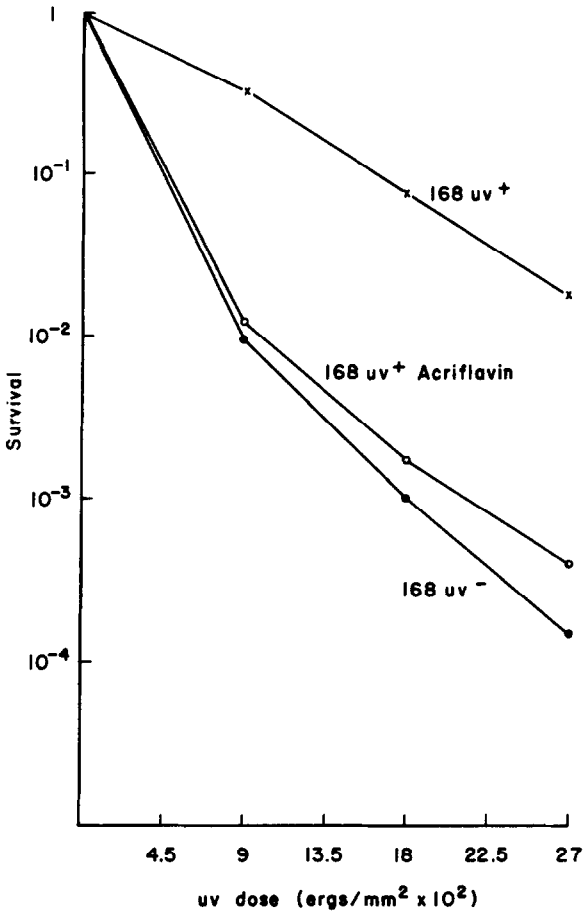

Fig. 2. Inhibition of host cell reactivation of phage SP3. SP3 at a concentration of 5 x 10^8 particles/ml was irradiated and plated on tryptone agar. (x) On host bacteria $168UV^+$ (o) on $168UV^-$ and (o) on $168UV^+$ in the presence of 0.5 ug/ml acriflavin.

tained when SP3 was plated on strain 168UV$^+$ and incubated in the presence of acriflavin, a dye known to inhibit DR (Witkin, 1961). These findings imply that the UV sensitivity of 168UV$^-$ cells is also due to some impairment of the DR system. HCR experiments were then carried out with phage SP82 which contains hydroxymethyl uracil (HMU) instead of thymine. The results were the same as those obtained with SP3, suggesting that the mechanism of repair cannot distinguish between HMU and thymine photo-products.

The survival of X-irradiated SP3 plated on strains 168UV$^+$ and 168UV$^-$ is shown in Fig. 3. No decrease in survival could be detected either on the UV$^-$

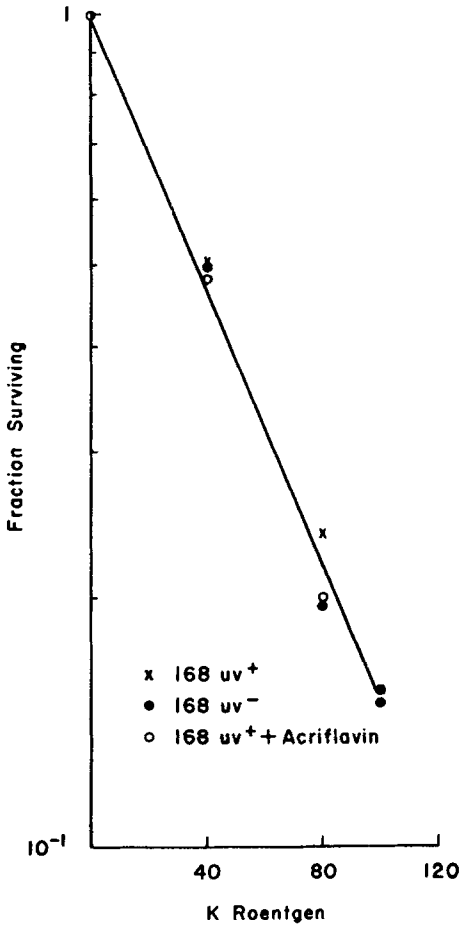

Fig. 3. Recovery of X-irradiated phage SP3 on strain 168UV$^-$ and on strain 168UV$^+$ with and without acriflavin. (x) 168UV+ host (o) 168UV$^+$ in the presence of 0.5 ug/ml of acriflavin (o) 168UV$^-$ host.

host or in the presence of acriflavin.

Transformation -- When competent 168UV$^-$ cells were exposed to UV irradiated DNA the number of transformants obtained was only slightly less than with 168UV$^+$ receptor cells. The possibility that DR may not function for intracellular transforming DNA was examined with irradiated erythromycin resistant (E^r) DNA and UV$^-$ and UV$^+$ tryptophan deficient receptor cells. Table I shows that the transformation efficiency of irradiated DNA is very similar whether competent

Table I

Recipient cells	Donor DNA	UV dose of DNA (ergs/mm^2)	Transformants x 10^2 E^r	T^+
168UV$^+$	UV$^+$	0	580	3000
	UV$^-$		620	3480
	UV$^+$	4×10^3	230	1490
	UV$^-$		217	1750
168UV$^-$	UV$^+$	0	520	4200
	UV$^-$		480	4160
	UV$^+$	4×10^3	190	770
	UV$^-$		160	830

UV$^+$ or UV$^-$ cells are used. It may also be noted that the number of transformants obtained from UV$^+$ and UV$^-$ DNA, with the radiation resistant receptor strain, indicates no enhanced radiation sensitivity of DNA extracted from strain 168UV$^-$.

Two possible explanations were considered to account for the lack of repair of irradiated transforming DNA in normally DR active cells. Competent cells may lack repair mechanisms or the structure of the intracellular transforming DNA may not be receptive to DR. Competent B. subtilis cells differ from non-competent cells in being completely non-photorestorable (Kelner, 1965). If competency represents a temporary inability of cells to effect any DR, then competent cells should be more UV sensitive than non-competent cells. To test

this possibility, strain SB-1A$^-$ was transformed with E^r DNA and irradiated 20 min. after addition of the DNA. The culture was plated on brain heart infusion agar for total survival and on selective transformation plates. Table II shows that at two UV doses the per cent survival of the total population was the same as the survival of three unlinked classes of transformants.

Table II

Resistance to Ultraviolet Irradiation of Competent and Non-Competent Cells

UV dose ergs/mm^2	Total viable count	Total number of transformants H$^+$	A$^+$	E^r
0	9×10^7	3.5×10^5	4.8×10^5	5.7×10^4
2.7×10^3	1.6×10^7 (17.5)*	5.2×10^4 (15)	7×10^4 (14)	6.9×10^3 (12)
4.05×10^3	1.4×10^6 (1.6)	1.4×10^4 (4)	1.5×10^4 (3.1)	5.6×10^3 (1)

*Numbers in parentheses represent per cent survival.

Linkage Studies -- To establish linkage of the UV sensitivity locus to known markers of the B. subtilis chromosome, transformation experiments were done with DNA extracted from a 168UV$^-$ strain resistant to bryamycin, erythromycin and micrococcin. SB-1A$^-$ and SB-25 were used as recipient strains. Colonies of transformants were grown to a cell density of 1×10^8, irradiated with a UV dose of 1350 ergs/mm^2, and plated on nutrient agar. Thirty colonies representing each marker were examined. Linkage could be detected only with the his_1 marker; five out of 30 transformants had simultaneously become histidine independent and radiation sensitive. The joint transformation of these markers did not increase when the transforming DNA was prepared and diluted by the procedures described by Kelly and Pritchard (1965) for the preservation of unstable linkage.

Discussion -- The ability of strain 168UV$^+$ to host cell reactivate irradiated SP3 and SP82 phages, compared to strain 168UV$^-$, points to the existence of a DR system in B. subtilis. As observed with radiation resistant strains of

E. coli, the action of the DR complex can also be inhibited by the presence of acriflavin.

Since 168UV$^-$ cells show no increased sensitivity to X-irradiation, general radiation sensitivity may not be a necessary consequence of DR impairment. Emmerson and Howard-Flanders (1965) pointed out that UV$^-$ strains of E. coli K12, which are unable to excise UV induced thymine dimers, can excise damage resulting from X-irradiation. The inability of acriflavin to inhibit the survival of X-irradiated SP3 offers additional evidence that DR may not be directly concerned with excision of X-ray damage.

The inhibition of DR in competent bacteria might be due to a general decrease in enzymatic activity of cells in this particular state, as suggested by Okubo and Romig (1965). If competent cells did lack DR enzymes, one would expect them to show a significant increase in UV sensitivity during this period. However, in contrast to the work of Okubo and Romig (1965) no difference in sensitivity of irradiated competent and non-competent cells could be detected. These findings corroborate experimental results described by Kelner (1964) for bryamycin transformants and histidine transformants (personal communication).

Since competent cells showed no increased UV sensitivity, the absence of DR of intracellular transforming DNA might be related to its secondary structure. The fact that transforming DNA per se is not refractive to DR has been demonstrated by Elder and Beers (1965), who achieved in vitro repair of Hemophilus influenzae DNA. If B. subtilis DNA becomes denatured following entry into competent cells, this may explain the lack of DR, since repair of ØX174 DNA occurs only in the double-strand, replicative form (Jansz, Pouwels and van Rotterdam, 1963; Yarus and Sinsheimer, 1964). The structure of intracellular B. subtilis DNA is still controversial. Bodmer and Ganesen (1964) and Pène and Romig (1964) report no evidence for the existence of denatured transforming DNA. However, Venema, Pritchard and Venema-Schröder (1965a, 1965b) suggest that the physical properties of transforming DNA shortly after uptake are those of single-strand molecules. Additional evidence for the intracellular state of B. subtilis transforming DNA is required to clarify this point.

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