EVIDENCE FOR EXTREME UV RESISTANCE OF MICROCOCCUS SP. NCTC 10785

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Summary : Micrococcus sp. NCTC 10785 isolated from irradiated Bombay duck (Harpodon nehereus) which was earlier found to be exceptionally resistant to gamma radiation, has also been shown to exhibit phenomenal resistance to ultraviolet (UV) radiation - higher than that reported so far for any other bacterium. The UV survival curve of this bacterium was found to be triphasic, comprising a very large shoulder extending up to 9,000 ergs/mm^2, an exponential part and a pronounced tail beginning at 25,000 ergs/mm^2 and extending well beyond 50,000 ergs/mm^2. The large shoulder presumably indicates operation of an extremely efficient repair mechanism. The distinct tailing effect is not due to genetic heterogeneity of population, since clones picked from the tail region showed the same triphasic response as the parental population. The type of tailing observed in the survival curve of this microorganism has not been shown previously for a bacterium exposed either to UV or ionizing radiation.

Studies with an orange-red pigmented tetracoccus* isolated from irradiated Bombay duck (Harpodon nehereus) indicated that this microorganism possessed exceptional resistance to gamma radiation (1). It was therefore pertinent to investigate whether this microorganism also displays phenomenal resistance to ultraviolet (UV) radiation.

In the present communication, evidence has been obtained to show that this microorganism withstands extremely high doses of UV radiation. The pronounced tail observed in the UV radiosurvival curve

*This culture has been deposited with the National Collection of Type cultures, Colindale, London and has been designated Micrococcus sp. NCTC 10785.

of this microorganism has not been reported before for any bacterial species exposed either to UV or ionizing radiation.

MATERIALS & METHODS

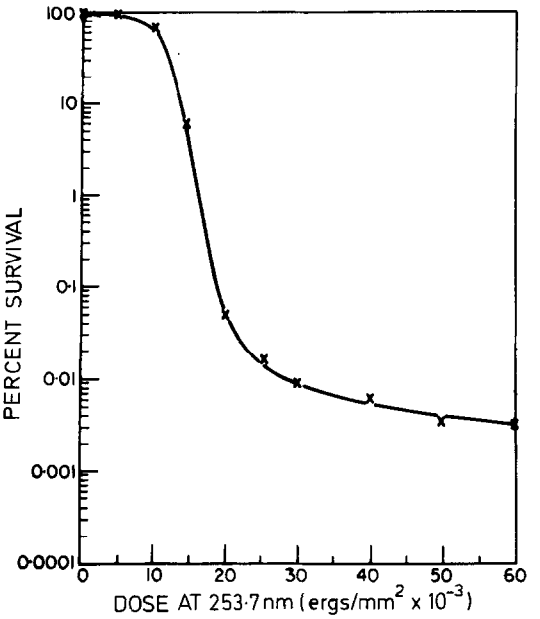
Culture : Micrococcus sp. NCTC 10785 isolated in this laboratory from irradiated Bombay duck (1) has been since maintained in this laboratory on TGYM slants.

Irradiation

18 hr old cell suspensions of Micrococcus sp. NCTC 10785 grown in TGYM medium at room temperature (28 - 30°C) on a shaker, were harvested, centrifuged, washed twice with 0.1 M phosphate buffer (pH 7.0) and resuspended in buffer solution to a concentration of approximately 10^7 cells/ml. Portions (5 ml) of the buffered cell suspension were pipetted into sterile petri dishes and exposed to UV radiation. A Philips TUV 15 W germicidal lamp equipped with a reflector was used, with 95% of its light output at 253.7 nm. Samples kept at a distance of 50 cms from the lamp were exposed to a dose rate of 15 ergs/mm^2/sec as measured by a thermocouple, and confirmed using the actinometry method (2). During irradiation the petri dishes were continuously rotated manually, to ensure uniform suspension of cells. Radiation survivors were recovered on TGYM agar. Colony counts were taken after 4 days incubation at 30°C.

RESULTS AND DISCUSSION

The radiation survival curve is depicted in Fig. 1. This curve is easily distinguishable from the UV survival curves of other bacteria, by the extremely large shoulder extending up to 9,000 ergs/mm^2 and a pronounced tail beginning at 25,000 ergs/mm^2 and extending well beyond



Legend to Fig. 1

Percentage survival of Micrococcus sp. NCTC 10785 to UV radiation. Each point represents the average value obtained from ten different experiments.

50,000 ergs/mm^2. Earlier studies (1) had shown that this bacterium was exceptionally resistant to gamma radiation and to possess biochemical characteristics distinct from Micrococcus radiodurans. Micrococcus sp. NCTC 10785 thus exhibits a very high degree of resistance not only to gamma radiation but also to UV radiation, a property also shared by M. radiodurans. The shoulder of the UV survival curve of Micrococcus sp. NCTC 10785 is more than twice as long as that reported for M. radiodurans (3) suggesting that this bacterium has a much more rapid and efficient repair mechanism than M. radiodurans.

Quinn et al (4) reported a tailing effect in gamma radiation survival curves of several Salmonella sp. irradiated in complex food material like shrimp and crab-meat. However, this tailing effect gradually dis-

appeared as the dilution was increased and it was therefore suggested by the authors that the shrimp and crab-meat possess protective properties. This phenomenon may therefore be considered as a pseudo-tailing effect and not an inherent characteristic of the cell. Masokhina-Porshnyakova and Ladukhina (5) also reported survival cruves with ill-defined tails for spores (10^8 - 10^9) of Clostridium botulinum suspended in beef and green peas and subjected to gamma radiation. The residual number of spores forming the tail was only 4 to 5 cells reaching in rare cases 20 cells, depending on the properties of the suspending medium.

The tail obtained after UV irradiation of cells of Micrococcus sp. NCTC 10785 comprised a significant fraction (approx. 0.01%) of the original cell population. The possibility that this reflects genetic heterogeneity in the cell population immediately suggests itself. However, this possibility is ruled out since clones picked from the tail showed the same triphasic UV response as the parental population. A somewhat parallel effect has been observed by Haynes (6) with E. coli B exposed to low doses of UV radiation - clones taken from the inflection in the radio-survival curve of this microorganism showed a similar UV response as the parental population, indicating absence of genetic heterogeneity. He has advanced evidence to show that this peculiar response is in fact due to phenotypic heterogeneity induced by UV radiation itself. According to him the inactivation of cells prior to the inflection is due to faulty coordination between various steps in excision repair and the tail component may be due to a UV-induced increase in the efficiency of the repair complex. However, such an explanation may not be applicable to the tailing effect observed in the UV survival curve of Micrococcus sp. NCTC 10785 which possesses an extensive shoulder.

The possibility exists that the tailing effect is the result of physiological differences among sub-populations in normal (genetically homogeneous) unirradiated cultures, but this seems unlikely considering the very large shoulder and high doses involved in the tail region.

Several workers have shown (7, 8) that UV-induced dimers between adjacent pyrimidine residues in DNA can account for a considerable fraction of the biological effects of UV radiation on DNA. Monomerization of these dimers either non-enzymatically (9) or enzymatically (10, 11) can lead to reversal of lethal effects in microorganisms and cause reactivation of UV-irradiated transforming DNA. The formation of cyclobutane type of pyrimidine dimers is a photochemically reversible reaction (12) as indicated by the equation :

$$\text{-PyPy-} \underset{\text{Kr}}{\overset{\text{Kf}}{\rightleftharpoons}} \overline{\text{-PyPy-}}$$

Thus dimers are constantly made and broken during irradiation, so that beyond a certain dose one may envisage the DNA polymer to be almost saturated with dimers and a steady-state distribution of dimers set up. The steady-state distribution of dimers may be obtained in vitro (12) using model polynucleotides or transforming DNA, but has not been shown previously to obtain in living cells since the cells would be totally inactivated prior to attainment of the steady-state.

All along the shoulder and exponential part of the UV survival curve of Micrococcus sp. NCTC 10785, Kf $>$ Kr and the cell inactivation observed may be attributed to the net amount of UV-induced dimers. However, at doses above 25,000 ergs/mm^2 it is highly probable that the steady-state distribution of dimers occurs (Kf = Kr) where the net amount of dimers produced is constant, any further kill due to increasing doses

being balanced by revival of cells resulting from dimer monomerization. The comparatively small increments in cell inactivation beyond 25,000 ergs/mm^2 may predominantly be due to irreversible photochemical reactions, yielding other products like cross-links, DNA-protein links or pyrimidine hydrates.

Further studies on the tailing phenomenon and on the effects of combined gamma and UV radiation on the survival patterns of Micrococcus sp. NCTC 10785 are in progress.

Whatever be the precise reasons for the distinct tailing effect observed in the UV dose survival curve of Micrococcus sp. NCTC 10785, this microorganism should undoubtedly be of considerable interest to radiobiologists for a more complete investigation of the molecular basis of repair mechanisms in cells.

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