Ultraviolet Radiation-sensitive Mutants of Micrococcus lysodeikticus

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Micrococcus lysodeikticus strain ATCC 4698 was treated with N-methyl-N'-nitro-N-nitroso-guanidine (NTG) according to the method of E. A. Adelberg, M. Mandel, and G. C. C. Chen (Biochem. Biophys. Res. Commun. 18:88, 1965). Before and after exposure to 100 μg of NTG per ml in tris(hydroxymethyl)aminomethane-maleic buffer (TM), pH 6, cultures were plated on tryptone, yeast extract, and glucose medium. Survival was 15% after 15 min and 2% after 60 min of exposure. Only chromogenic-type mutants could be detected on this medium. The mutant colonies observed ranged in color from off-white to pale pink and pale orange as compared with the yellow parental type. Their frequency among the survivors in the treated population was around 0.5%. Such mutants are not frequently encountered in routine handling of M. lysodeikticus, and none were found among untreated controls. Since their occurrence was markedly increased after exposure to NTG, NTG appeared to be an effective mutagen for chromogenicity; therefore, additional types of mutants were sought among the survivors. This report deals mainly with the isolation and characterization of ultraviolet (UV) radiation-sensitive mutants.

Plates spotted with overnight cultures of recloned colonies were prepared in triplicate and irradiated with UV for 0, 180, and 240 sec, respectively, and were examined for growth after 72 hr of incubation at 37°C. Of 41 strains isolated from an NTG-treated suspension, 2 showed no visible growth after 180 and 240 sec irradiation, whereas the others showed the same confluent growth as unirradiated controls. Since 1 UV-sensitive mutant was also found among 29 strains isolated from the same suspension before the addition of NTG, the other 2 may have been fortuitous or the TM buffer may have had an effect. In any case, further testing is required to determine the frequency of spontaneously occurring UV-sensitive mutants before the mutagenic effect of NTG can be assessed with certainty for this trait in M. lysodeikticus.

UV-sensitive mutant strains were assayed for survival after successive doses of UV radiation and also for their ability to repair UV-damaged bacteriophage when used as unirradiated hosts. The strains of M. lysodeikticus used were: 4698, yellow parental; 4698-1, yellow recloned from 4698; 4698-52, yellow, isolated from a culture prior to NTG treatment; 4698-4, pink, and 4698-33, yellow, both isolated from the same culture as strain 52 after NTG treatment; and 4698-33-1, pink, isolated as a survivor of UV-irradiated strain 33. Bacteriophage N1 was obtained through the courtesy of H. B. Naylor and has been described by J. V. Scaletti and H. B. Naylor (J. Bacteriol. 78:422, 1959). The parental 4698 strain was used in preparation of a fresh broth lysate of the phage.

The broth medium used throughout these experiments contained 1.0% tryptone, 0.5% yeast extract, 0.1% glucose, and 0.5% NaCl. Agar, 0.7 or 1.5%, was added as needed; 0.067 M phosphate buffer, (pH 7) was used for bacteria and phage suspensions prior to irradiation and plating. A General Electric germicidal lamp was used at a distance of 56 cm and the dose rate was 8 ergs per mm² per sec. All suspensions were aged during irradiation. Broth cultures of the bacteria were grown for 18 hr at 37°C (20 ml in 125-ml flasks) without aeration. They were centrifuged and resuspended in buffer prior to irradiation. Bacterial counts were made after spreading on 1.5% agar and incubating at 30°C for 72 hr. Phage was diluted and irradiated in buffer and plated with 18-hr broth cultures by the usual soft-agar overlay method; plaque counts were made after incubation at 30°C for 48 hr. All experiments were carried out in dim light to avoid possible photoreactivation.

The UV survival curves of the bacterial strains are shown in Fig. 1. The curves of strains 4698 and 4698-1 have distinct shoulders, and these strains may be considered UV-resistant (R. H. Haynes, Photochem. Photobiol. 3:429, 1964). By comparison, strains 52, 4, 33, and 33-1 (the last two with coinciding curves) are UV-sensitive, but not all to the same extent. When the cultures were grown with aeration for 4 to 5 hr before
assay and when the incubation temperature for survival after irradiation was 37°C, the survival curve of each strain was essentially the same as shown in Fig. 1.

All the strains used were susceptible to N1 phage and the efficiency of plating of unirradiated phage did not vary significantly on any. Figure 2 shows the survival of UV-irradiated N1 phage when assayed on different unirradiated host strains. The ability to repair or propagate UV-damaged phage, usually designated as host cell reactivation (hcr), was impaired in the UV-sensitive strains 33 and 33-1 and to a lesser degree, and perhaps in a different way, in 52. Strain 4, however, although more UV-sensitive than 52, repaired damaged phage to the same extent as did the parent strain 4698. A host effect on the survival of UV-damaged phage is clearly indicated for M. lysodeikticus and is similar to that described for Escherichia coli strain B and its UV-sensitive mutants on irradiated T1 phage (S. A. Ellison, R. R. Feiner, and R. F. Hill, Virology 11:294, 1960; R. F. Hill and R. R. Feiner, J. Gen. Microbiol. 35:105, 1964).

With regard to UV sensitivity and hcr, the strains thus far derived from M. lysodeikticus 4698 fall into three groups: UV resistant and hcr positive (4698 and 1); UV sensitive and hcr negative (52, 33, and 33-1); and UV sensitive and hcr positive (4). These mutants may be of interest in a comparative study of pyrimidine dimer formation and excision such as occurs in the highly UV-resistant M. radiodurans (M. E. Boling and J. K. Setlow, Biochim. Biophys. Acta 123:26, 1966) and also of enzymes, extracted from M. lysodeikticus, which can repair UV-damaged DNA in the dark (R. L. Elder and R. F. Beers, Jr., J. Bacteriol. 90:681, 1965).