APPLIED MICROBIOLOGY, July 1974, p. 151-153

Vol. 28, No. 1
Printed in U.S.A.

NOTES

Response of *Mycobacterium avium* to Ultraviolet Irradiation

C. M. McCARTHY AND J. O. SCHAEFER
Department of Biology, New Mexico State University, Las Cruces, New Mexico 88003

Received for publication 21 February 1974

The survival response of *Mycobacterium avium* to ultraviolet irradiation demonstrated the presence of a photoreactivating repair system.

Mycobacterium avium, a member of Runyon's Group III mycobacteria (4), has been shown to be the causative agent of human lung disease which clinically resembles tuberculosis. *M. avium* has an apparent slow rate of growth, and an incubation period of 2 to 3 weeks is required for maximal numbers of colonies to develop on an agar plate. The organism exhibits a growth cycle in which small cells actively engage in protein synthesis and elongate into filaments which then undergo rapid fragmentation to form coccobacilli (3). Thus, it is possible to synchronize *M. avium* in its growth cycle by selecting for small, uniformly sized cells, which do not pass a 0.8-μm filter but do pass a 1.2-μm filter. We examined the ultraviolet sensitivity of such synchronized cells of *M. avium* in an effort to estimate the number of sensitive targets per cell.

DM 9, a pathogenic isolate of *M. avium*, was grown, and cells of uniform size were obtained by selective filtration as described previously (3). These cells were irradiated with ultraviolet light for timed intervals from 0 to 240 s at 0.5 μW (5 erg/s) intensity and were plated for colony-forming units (CFU) on 7H10 agar (BBL). The results, as percent survival at different doses, are shown in Fig. 1. Due to the sloping shoulder from 0 to 300 ergs/mm^2, the effective inactivation dose range was usually from 300 to 1,200 ergs/mm^2, and at these dosages inactivation did not occur at a constant rate (Fig. 1). The reasons for the multitarget response are not known. The cells were predominantly single rods with some diplobacilli present, but there were no aggregates of cells. Back-extrapolation to the ordinate did not provide a reasonable estimate of the number of sensitive targets per cell. The 14-day postexposure incubation on plates could conceivably have allowed extensive dark repair to occur, or, conversely, the cells might not have been active metabolically at the time of irradiation, which could have caused the resistance shoulder.

An effective photorepair system is present in *M. avium*, because a period of 24-h postirradiation exposure to visible light greatly increased survival (Fig. 2). The greatest increase in cell survival was for a dosage of 900 ergs/mm^2 for which there was a survival increase from 2.3×10^4 CFU/ml in the dark control (Fig. 2B) to 7×10^6 CFU/ml for the cultures exposed to light (Fig. 2A).

The photoreactivation kinetics after a single dosage of ultraviolet light at 900 ergs/mm^2 are shown in Fig. 3. CFUs doubled after only 30 min and reached a maximum after 3 h of visible light exposure. This demonstrates that the selected small cells were active metabolically after irradiation and suggests that the resistance shoulder (Fig. 1 and Fig. 2B) was due to dark repair.

David (1), in comparing ultraviolet radiation inactivation of mycobacteria, reported that *M. avium-intracellulare* yielded a resistance shoulder of 400 ergs/mm^2 and constant inactivation thereafter with increasing dosages. In order to kill 99% of the *M. avium* cells, David (1) used 1,400 ergs/mm^2 in contrast to the 700 ergs/mm^2 reported here. Such variation in results may be because he did not select for cells of uniform size or possibly because of microbial strain differences in sensitivity to ultraviolet light. Photoreactivation was reported for *Mycobacterium smegmatis* (5), and David (1) showed that *M. avium* can undergo photoreactivation with kinetics similar to those reported here (Fig. 2A). We found, however, that the level of inactivation by ultraviolet radiation affected the efficiency of photoreactivation (Fig. 2). *M. avium*

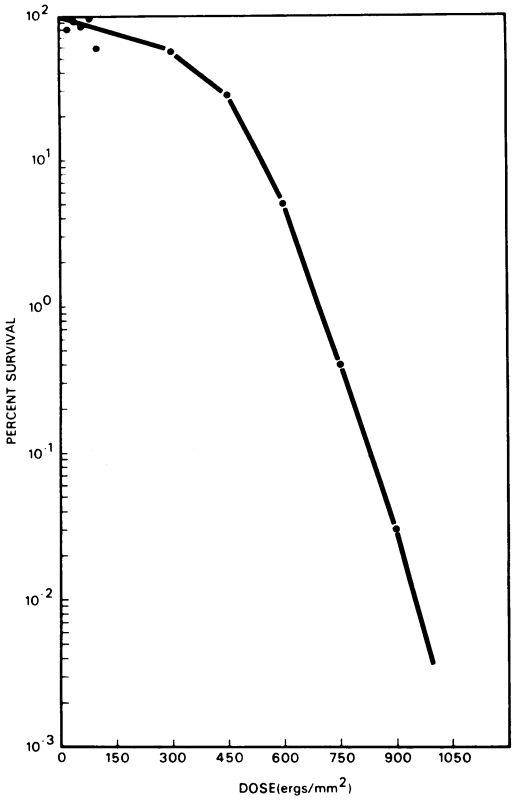

FIG. 1. *Survival of ultraviolet-irradiated M. avium cells. Frozen cells of DM 9, a pathogenic variant of M. avium, were thawed and diluted into Medium B which contained B salts (2), 0.5% glycerol, 0.5% dialyzed bovine albumin (2), 0.01% oleic acid, and 0.1% Tween 80. The cells were incubated on a rotary water bath shaker at 125 rpm and 37 C for 5 or 6 days to a density of 5 × 10⁸ CFU/ml. A volume of 30 ml was passed through a 0.8-μm filter (Millipore Corp.). The collected cells were resuspended in 12 ml of B salts with 0.1% Tween 80 and 0.5% glycerol. The suspension was vortexed vigorously and then passed through a 1.2-μm filter. A 5-ml volume of the 1.2-μm filtrate (1.5 × 10⁸ CFU/ml) was placed in a thick-bottomed petri dish and irradiated with ultraviolet light. At timed intervals, a 0.1-ml sample was withdrawn, diluted, and plated on 7H10 agar. The plates were placed in plastic bags and incubated for 14 days at 37 C in the dark. The ultraviolet source was a General Electric (G.E.) short-wave germicidal ultraviolet lamp (G 8T 5). Irradiation intensity was measured with a Blak Ray ultraviolet dosimeter. The intensity level was set at 0.5 μW/mm², and a timer was used to monitor dosage increments. The cell suspension was mixed on a rotary shaker at 100 rpm during irradiation. As a precaution against photoreactivation, all operations were conducted in a dark room illuminated with a G.E. "Bug" lamp. CFUs were expressed as percent survival of the unirradiated population.*

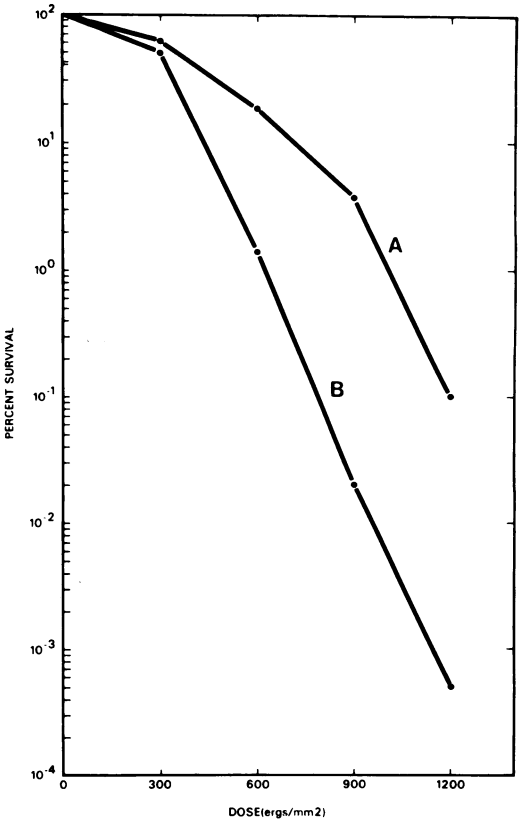

FIG. 2. *Photoreactivation of ultraviolet-irradiated M. avium cells. The cells were filtered, irradiated, and plated as described for Fig. 1. A G.E. white light bulb (100 lux; F 8T 5W) was employed to illuminate the surface of the agar in plastic petri dishes which had been inoculated by spreading appropriate dilutions of the irradiated cells. The plates were exposed to the light at a distance of 45 cm at 37 C for 24 h (A). The light did not increase the temperature, because constant circulation of air was maintained by a fan. A thermometer placed by the plates indicated that no temperature change occurred. After white light exposure, the plates were incubated for the remainder of the 14-day incubation period at 37 C in the dark. Control plates (B) similarly inoculated with irradiated cells were maintained in the same incubator, but were covered with aluminum foil during the light exposure process.*

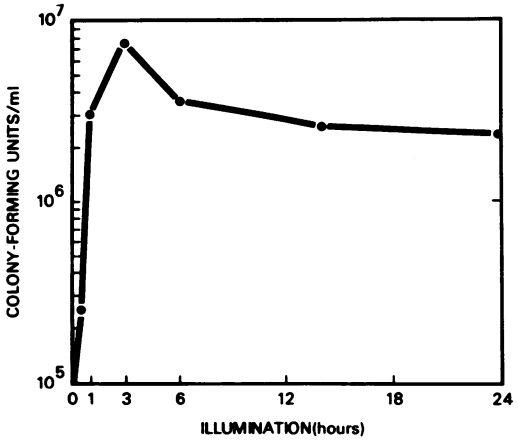

FIG. 3. *Kinetics of photoreactivation in M. avium. The procedure was identical to that described for Fig. 2, except that the experimental plates were exposed to white light for the designated time periods after a single dose of 900 ergs/mm².*

cells, incubated in the dark for 0, 1, 3, or 6 h after ultraviolet radiation, were all photoreactivable to the same extent. Thus, these cells may respond positively to visible light long after exposure to ultraviolet light. Clinical laboratories using ultraviolet light as a disinfectant may be concerned with the counter-influence of white light on the inactivation of *M. avium* and other mycobacteria by ultraviolet irradiation.

This investigation was supported by Public Health Service grant AI-11171-02 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. David, H. L. 1973. Response of mycobacteria to ultraviolet light radiation. Amer. Rev. Resp. Dis. **108**:1175-1185.
2. McCarthy, C. 1970. Spontaneous and induced mutation in *Mycobacterium avium*. Infect. Immunity **2**:223-228.
3. McCarthy, C. 1971. Electronic counting in growth studies of *Mycobacterium avium*. Appl. Microbiol. **22**:546-551.
4. Runyon, E. H. 1965. Pathogenic mycobacteria. Advan. Tuberc. Res. **14**:235-287.
5. Sellers, M., R. Nakamura, and T. Tokunaga. 1970. The effects of ultraviolet irradiation on mycobacteriophages and their infectious DNAs. J. Gen. Virol. **7**:233-247.