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Simple Method to Demonstrate Radiation-Inducible Radiation Resistance in Microbial Cells†

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A simple method for detection of radiation-inducible radiation resistance was developed by irradiating aliquots (0.01 ml) of cell suspension on agar plates. Part of each experimental plate was subjected to an induction treatment, and subsequent radiation resistance was compared with that of untreated cells on the same plate. The UV radiation resistance of a *Micrococcus* sp. was increased approximately 1.6 times by an induction treatment. This simple procedure of irradiating cells in a "fixed" position on agar avoided washing, centrifugation, and cell enumeration required in traditional methods.

A common approach for studying a mutagen was developed by Pollard and Achey (2). Their procedure involved treatment of cells with inducing agent followed by incubation for about 50 min, allowing protein synthesis, which was then blocked by treatment with rifampin or chloramphenicol. Following the induction treatment, the cells were exposed to graded doses of the mutagen. Growth, expressed as colony-forming ability, was determined by plating and comparison with a control sample. The need for frequent washing and centrifugation made this procedure tedious.

To determine the presence of inducible resistance in a radiation-resistant *Micrococcus* sp., we devised a simple system without a tedious washing operation. This system can be termed an agar method, because throughout the study cells under test are "fixed" on an agar plate. This report describes the system and shows the effects of some variations in experimental conditions.

MATERIALS AND METHODS

Organism and media. Isolate C-7 cells, *Micrococcus* sp. (3), were propagated in plate count broth at 32°C. Plate count agar plates were prepared with appropriate dryness to absorb the cell suspension carrier, leaving the cells adsorbed on the surface (4).

UV irradiation. UV irradiation was carried out at room temperature, using a General Electric G15T8 germicidal lamp at a distance of 33 cm from the test sample, giving an incident dose rate of 20 ergs mm^{-2} s^{-1}. In this report doses are expressed in minutes.

General procedure of the agar method. Aliquots of 0.01 ml of cell suspension were inoculated onto plate count agar plates and allowed to be absorbed at room temperature, producing cell films about 8 mm in diameter. Some of the cell films were irradiated at a low dose as an induction treatment; others were used as controls. After incubation to complete the induction process, plates were treated with graded doses of radiation. Subsequent incubation allowed survivors in the films to grow and form a cell mass when the surviving fractions were high or form discrete colonies when the surviving fractions were low. Thus, a simple visual comparison provided a guide to evaluating the effectiveness of the induction treatment. Cells under investigation were always fixed on agar plates throughout the successive steps: induction irradiation, postinduction incubation, challenging irradiation, and final incubation for the survivors to grow.

Quantitative expression of results. Two parameters, D_c and F_i, are used to describe the results quantitatively. D_c is the dose required to inactivate a majority of the population in a "film" so that only a "countable" number of CFU, 30 ± 10, is shown. F_i, induction factor, is defined by the following equation: $F_i = D_c$ for population with induction treatment/D_c for population without induction treatment. Thus, $F_i > 1$ indicates occurrence of induction under test conditions.

RESULTS

UV-inducible radiation resistance in isolate C-7. Each trial consisted of eight prepoured and dried plate count agar plates, which were divided into halves by external marking on the bottom (Fig. 1a). Cells of C-7 in the exponential growth phase (ca. 10^7 CFU ml^{-1}) were pipetted onto the plates, with each plate receiving six 0.01-ml aliquots (Fig. 1b). The inoculum was allowed to absorb into the agar at room temperature for 40 min before initial exposure to UV irradiation for 1 min while half of each plate was shielded (Fig. 1c). After incubation at 32°C for 40 min, the plates were treated with graded doses of UV radiation (0 to 9 min). The plates were evaluated for the extent of growth after incubation at 32°C for 36 h. Figure 2 shows the qualitative effect of an induction treatment on subsequent survival of cells to a challenging dose of 5 min. The quantitative effect of induced resistance was shown by F_i values of 1.5 to 1.8.

Effect of induction dose level of UV radiation on subsequent UV radiation resistance. Three induction doses (0.5, 1.0, and 1.5 min) of UV radiation were compared in resulting F_i values. The experimental conditions were the same as those in the previous section except for the various preirradiation doses. In the first two trials, all treatments showed an F_i value of 9/7; i.e., D_c without preexposure = 7 min and D_c with preexposure = 9 min. In the second trial all treatments resulted in an F_i value of 9/6. The results indicated that the

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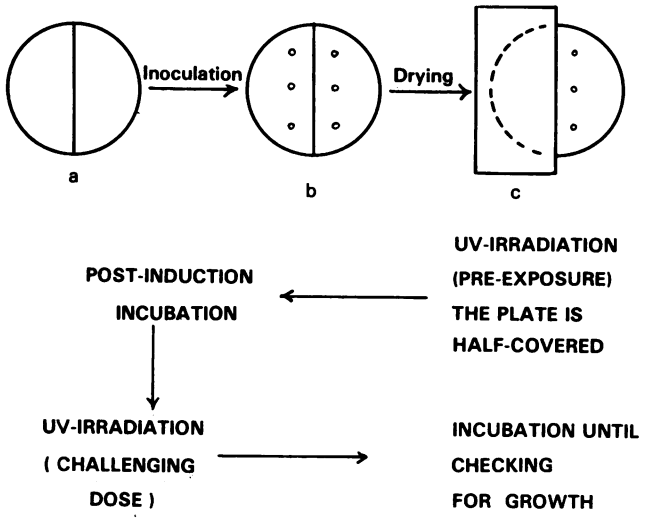

FIG. 1. Scheme of an agar system for studying radiation-induced radiation resistance in microbial cells.

level of induced survival was constant for induction doses ranging from 0.5 to 1.5 min.

Effect of postinduction incubation on magnitude of UV-inducible radiation resistance. With a fixed induction dose level of UV radiation (1 min), various postinduction incubation times were compared in the resulting F_i values. An incubation time of 20 min or more was required for an observable increased resistance and an incubation period of ca. 40 min provided the maximum inducible resistance (Fig. 3).

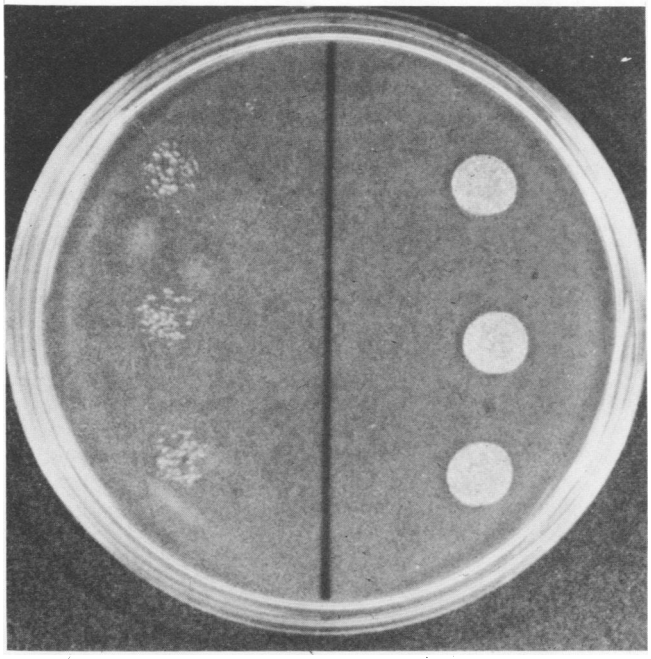

FIG. 2. Example plate showing difference in suryival between preexposed (right) and non-preexposed (left) samples. Inducing dose was 1 min; postinduction incubation time was 40 min; and challenging dose was 5 min (referred to in Fig. 1).

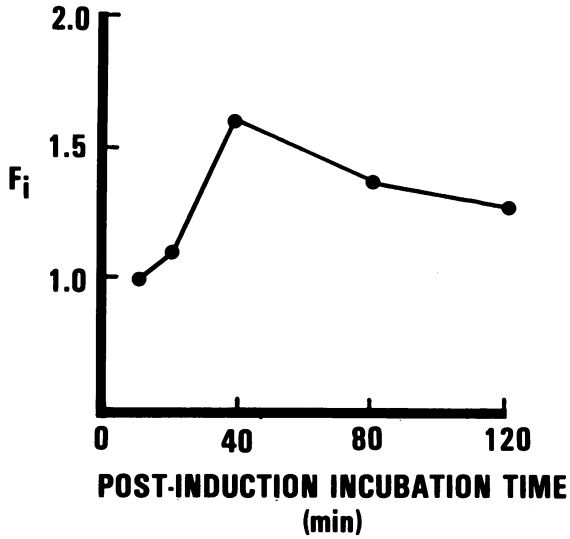

FIG. 3. Effect of postinduction incubation time on plate count agar (32°C) on induction factor (F_i) for isolate C-7 cells at the exponential growth phase.

DISCUSSION

The agar plate method for observing induced radiation resistance is a simple, definitive way to demonstrate changes in the microflora. This method avoids the previously utilized washing treatment, which potentially influences the physiological condition of the cells.

Through use of the new method, a low dose of UV radiation plus an incubation treatment was shown to increase the subsequent resistance of a radiation-resistant *Micrococcus* sp. This response was probably within the shoulder of the death curve of this highly radiation-resistant microorganism (3). The extent of induction and the increased radiation resistance were relatively uniform for induction doses ranging from 0.5 to 1.5 min. The constant induction level implied that an induction period of 0.5 min of UV irradiation "saturated" the inducible factor. The degree of induction may have been limited by a factor other than the quantity of UV radiation, e.g., availability of a substrate for synthesis of a protein.

The postinduction incubation time appeared to influence the inducible level of radiation resistance in these cells (Fig. 3). Maximum resistance induction occurred after approximately 40 min of postinduction incubation, which is nearly two-thirds the generation time of the cells in the exponential growth phase (about 65 min in plate count broth) (3). This is approximately the same induction period reported by Keller et al. (1) for a radiation-resistant *Moraxella-Acinetobacter* sp.

The simplicity of the agar method should facilitate study of the phenomenon of inducible radiation resistance and perhaps the mechanism of action. For example, use of this method in our laboratory has demonstrated that preexposure to UV radiation did not increase gamma radiation resistance of C-7 cells (i.e., $F_i \leq 1$). However, preexposure to 50 krads of gamma radiation made similar cells much more resistant to UV radiation (F_i value of approximately 2).

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