Photoreactivation of *Pseudomonas cepacia* after Ultraviolet Exposure: a Potential Source of Contamination in Ultraviolet-Treated Waters

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Cells of a naturally occurring strain of *Pseudomonas cepacia* grown in distilled water were exposed to ultraviolet radiation. Irradiated samples incubated on membrane filters or in fluid recovery media in the absence of light showed no evidence of dark repair mechanisms. When samples were exposed to fluorescent light ranging from 50 to 950 foot candles (538 to 10,222 lux) of illumination, apparent photo-induced repair of ultraviolet injury resulted in 1- to 4-log increases in viable cells recovered.

Water treated to remove chemical impurities (i.e., distillation, ion exchange, or reverse osmosis) is widely used in medical applications in pharmaceutical preparations or in special units such as respiratory therapy and hemodialysis (4, 7, 10). However, in the absence of disinfectants, contamination with gram-negative bacteria capable of multiplying rapidly and reaching high levels (1, 2, 6, 9, 14) can constitute a potential health hazard in the hospital environment (7, 11-13). Ultraviolet (UV) irradiation has been reported as a simple and reliable means of inactivating microorganisms in industrial and potable water supplies (14, 15). Consequently, it was of interest to us to study the efficacy of this technique against waterborne gram-negative organisms frequently implicated in nosocomial infections.

In initial tests with a commercial UV unit obtained on loan from the manufacturer, viable counts of naturally occurring cells of *Pseudomonas aeruginosa* (6) and other pseudomonads in contaminated distilled water exposed to UV were effectively reduced by 99.99%. However, results obtained with a naturally occurring strain of *Pseudomonas cepacia* (2) suggested that photoreactivation of UV-damaged cells might have occurred, and further tests were designed to explore this phenomenon.

MATERIALS AND METHODS

Cultures. Glass carboys containing approximately 20 liters of laboratory distilled water were autoclaved at 121 C for 6 h, cooled to room temperature (22 C), and inoculated with 10 ml of naturally occurring cells of *P. cepacia* maintained in distilled water (2). Carboys were incubated for 5 to 7 days at 37 C, resulting in levels of 10^5 to 10^6 cells/ml. Prior to testing, the contents of each carboy were aseptically mixed with magnetic stirrers to achieve uniform distribution of the cells.

Irradiation and assay procedures. An Aquafine water sterilizer (model SL-10A, Aquafine Corp., Burbank, Calif.), rated as providing in excess of 25,000 microwatt seconds of germicidal energy (2,537 Å) per cm^2 and having a maximum design flow rate of 15 liters/min was used. Contents of the carboys were siphoned through the UV chamber at a flow rate of 11.4 liters/min, providing a theoretical 7.1 s of UV exposure. Effluent from the unit was aseptically collected at 30-s intervals in sterile screw-capped bottles covered with aluminum foil; all tests were conducted in a vertical laminar flow clean room to minimize extraneous contamination. Samples were assayed using the membrane filtration technique; filters (0.45-μm pore size, Millipore Corp.) were placed on standard methods agar (Difco) in 60- by 15-mm petri dishes (Falcon Plastics, Oxnard, Calif.) and incubated at 37 or 22 C for 48 h. In one series of experiments water agar (laboratory distilled water with 1.5% Ionagar no. 2 [CoLab Laboratories]) was also employed to compare the effect of the recovery medium. Filters incubated without exposure to reactivating light (dark grown) were placed immediately in baskets lined with aluminum foil. To assess the effect of exposure to various light intensities on recovery of irradiated cells, filters were incubated for 48 h under fluorescent light (Westinghouse cool white, high output) providing approximately 50, 600, or 950 footcandles (538, 6,456, or 10,222 lux) of illumination (Weston illumination meter, model 756, Quartz filter; Weston Electric Instrument Corp., Newark, N.J.). To determine whether the length of exposure influenced degree of recovery, replicate samples of 950 footcandles were exposed for only 30 min and then dark grown to 48 h. Viable counts of control unirradiated samples were determined from filters incubated under each of the conditions described above.

To assess possible effects of liquid holding recovery (3), 50-ml aliquots of irradiated samples of naturally

occurring cells were dispensed into sterile Erlenmeyer flasks and into flasks containing 50 ml of exhausted dialysate which had been collected during a hemodialysis procedure and sterilized by membrane filtration. Previous experiments have demonstrated (7) an increased growth potential of waterborne gram-negative bacteria in dialysate fluids. In view of the possible application of UV treatment in controlling contamination problems in hemodialysis units, it was of interest to determine whether these fluids might also influence photoreactivation. For dark-grown incubation, flasks also were placed in aluminum foil-lined baskets and incubated at 37 C for 48 h. Light-grown

samples were exposed to fluorescent light (950 footcandles) either for 30 min and then dark grown or continuously for 48 h. Total viable counts of irradiated and control samples were determined using standard methods agar pour plates.

RESULTS AND DISCUSSION

Exposure to UV radiation effectively reduced viable counts of naturally occurring cell suspensions 6 to 8 logs. In assessing the effect of the recovery medium itself using membrane filtration techniques (Table 1), dark-grown filters showed no viable cells in 100-ml samples plated either on standard methods or water agar. Filters which were light exposed (950 footcandles) and then dark grown showed 2-log increases in viable counts, with comparable recoveries on standard methods and water agars. With continuous light exposure additional 2-log increases in viable counts were observed. In fluid media (Table 2) control unirradiated cells in laboratory distilled water increased from <1/ml to 10^5 cells/ml in 24 h. Irradiated cells grown in the absence of fluorescent light showed no recovery in a 24-h period, even with the addition of dialysate containing carbon and nitrogen wastes; samples taken at 48 and 72 h still showed no viable cells present. However, even a 30-min exposure followed by dark incubation allowed some recovery and subsequent growth of presumably injured cells. Samples continuously exposed to light showed 3-log increases in viable counts within 2 h, far greater than would be expected from growth alone in these types of menstrua (2, 6, 7). Although the type of fluid medium did not appear to affect

TABLE 1. *Factors affecting comparative recovery of naturally occurring cells of P. cepacia on membrane filters after UV exposure in distilled water[a]*

Sample	No. of *P. cepacia*/100 ml					
	Dark grown[b]		Light exposed, dark grown[c]		Light grown[d]	
	SM agar	Water agar	SM agar	Water agar	SM agar	Water agar
1	0	0	700	770	67,900	66,200
2	0	0	260	472	20,000	34,200

[a] Viable counts prior to exposure = 1.2 × 10^7 cells/100 ml. SM agar, Standard methods agar (Difco).

[b] Filters were incubated in the absence of light at 37 C for 48 h.

[c] Filters were exposed to cool white fluorescent light (~950 footcandles) for 30 min and then dark grown at 37 C to 48 h.

[d] Filters were exposed continuously for 48 h to cool white fluorescent light (~950 footcandles) at 37 C.

TABLE 2. *Factors affecting comparative recovery of naturally occurring cells of P. cepacia in fluid media after UV exposure in distilled water[a]*

Incubation at 37 C (h)	No. of *P. cepacia*/ml							
	Unirradiated cells in LDW[b]		Irradiated cells in LDW			Irradiated cells in LDW with added dialysate		
	Dark grown[c]	Light grown[d]	Dark grown	Light exposed, dark grown[e]	Light grown	Dark grown	Light exposed, dark grown	Light grown
0	0.2	0.5	0	0	0	0	0	0
0.5				3.5			1	
1					100			20
2	0.2	0.5	0	6	1,500	0	3	1,040
5	6	13	0	11	10,000	0	9	10,800
24	390,000	310,000	0	38,000	2,900,000	0	90,000	75,000,000

[a] Viable counts prior to exposure = 1.2 × 10^5 cells/ml.

[b] LDW, Laboratory distilled water.

[c] Incubated in the absence of light at 37 C for 24 h.

[d] Exposed continuously for 24 h to cool white fluorescent light (~950 footcandles).

[e] Exposed to cool white fluorescent light (~950 footcandles) for 30 min and then dark grown at 37 C for 24 h.

recovery itself, the differences in total population levels at 24 h did appear to reflect the greater nutrient content of dialysate fluids.

The results of four tests conducted at each of three levels of continuous illumination during 48 h of incubation showed the mean recovery value at 600 footcandles to be slightly higher than the mean value at either 50 or 950 footcandles. The differences were not statistically significant. Results presented in Table 1 suggested that length of exposure to fluorescent light was a major factor in reactivation. However, in a total of five tests comparing the effect of 30-min exposure to 950 footcandles illumination with the effect of 48 h of exposure, the results were variable and the difference between mean recovery values was not statistically significant. A most noteworthy finding in this investigation was that the mean recovery value for each of the exposure categories tested was significantly higher than the mean dark-grown control values ($P < .01$).

The use of UV irradiation does afford promise in controlling contamination by waterborne gram-negative organisms. However, the results reported here, in which photoreactivation occurred even under average room light conditions, reaffirm the need for caution in assessing UV effects against specific microorganisms. Both photoreactivation and dark repair mechanisms have been described in a variety of microorganisms other than *Pseudomonas* (5, 8). In hospital environments, particularly where UV-treated water might be stored or recirculated prior to use in the absence of a disinfectant, such mechanisms could allow for growth of survivors, leading to subsequent high levels of microbial contamination in water supplies assumed to contain few or no viable microorganisms.

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