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Resistance of Selected Strains of *Pseudomonas aeruginosa* to Low-Intensity Ultraviolet Radiation

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The resistances of 10 strains of *Pseudomonas aeruginosa* and other microorganisms to an ultraviolet (UV) intensity of 100 $\mu W/cm^2$ were determined. Organisms were exposed in 2- or 15-ml saline suspensions contained in uncapped polyethylene bottles for increasing periods of time, and the surviving fractions were enumerated. Decimal reduction times were calculated by regression analysis, using the least-squares method. The 10 strains of *P. aeruginosa*, compared with *Micrococcus radiodurans* and *Candida albicans*, were very susceptible to low-intensity UV radiation. Results from this study showed that a UV intensity of 100 $\mu W/cm^2$ penetrated saline suspensions up to 40 mm deep sufficiently to kill high levels of microbial cells, especially *P. aeruginosa* cells. These results allowed us to design a system for determining and monitoring the sterilization capability of low-intensity UV radiation. In our particular case, UV proved to be an efficient mode for sterilizing saline suspensions of *P. aeruginosa* in polyethylene bottles. The significance and application of these findings with regard to supporting UV as a sterilant are discussed.

Pseudomonas aeruginosa has been involved in the contamination of ophthalmic pharmaceuticals, and severe ocular infections have occurred as a result of using these medicaments (4, 5, 17). *P. aeruginosa* is a major contaminant of water used in the preparation of pharmaceutical products (3, 13, 19). Favero et al. (8) have shown that *P. aeruginosa* is capable of growth in filter-sterilized distilled water. This organism can cause devastating infections of debilitated and traumatized eyes (9, 10); consequently, *P. aeruginosa* is of utmost concern to manufacturers of ophthalmic products.

Ultraviolet (UV) radiation has been used effectively for sterilizing air (12, 15, 16, 20), vaccines (6, 11), and water (14, 16, 18, 21). The literature contains numerous reports concerning the lethal effect of UV radiation on microorganisms, but none have dealt extensively with a variety of species and strains of *Pseudomonas* sufficient to satisfy our needs. The following study was conducted to obtain dose-survivor profiles for selected organisms, especially *P. aeruginosa*. This information was used to design a system for evaluating the use of low-intensity UV as a sterilizing mode for destroying high levels of *P. aeruginosa* contained in polyethylene bottles.

MATERIALS AND METHODS

Test organisms. *P. aeruginosa* strains ATCC 9027, ATCC 10145, ATCC 14207, ATCC 15442, and ATCC 27853, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 10541, *Micrococcus radiodurans* ATCC 13939, *Candida albicans* ATCC 10231, *Pseudomonas diminuta* strains ATCC 11568 and 19146, *Pseudomonas maltophilia* ATCC 13637, *Pseudomonas cepacia* ATCC 25416, and *Pseudomonas putrefaciens* ATCC 8071 were obtained originally from the American Type Culture Collection, Rockville, Md. *P. aeruginosa* strains B-2, G-2, BS-4, WB-1, and SH-2918 were clinical isolates from eye, ear, and burn infections. These organisms were obtained from the stock culture collection at Alcon Laboratories, Inc., Forth Worth, Tex. All organisms were subcultured at 7-day intervals and maintained at 4°C on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants.

Preparation of suspensions. Trypticase soy agar slants were streaked with test cultures and incubated at 32 to 35°C for 12 to 18 h before each exposure study. Cells were harvested with sterile 0.85% saline into sterile cuvettes (18 by 150 mm), and the turbidity of each suspension was adjusted to an optical density at 525 nm of 0.3 (50% transmittance) on a Spectronic 20 spectrophotometer. Viable cell counts of these suspensions were determined, and appropriate dilutions were prepared as needed to obtain the desired number of cells for each radiation study. Immediately before each study, suitable cell suspensions were made with 0.85% saline in 1,000-ml quantities contained in glass flasks and mixed for 15 min on a magnetic stirrer.

Irradiation procedures. The UV energy source was a 17-W Westinghouse low-pressure mercury "germicidal" lamp (Sterilamp, model 782L-30). The lamp was installed in a cabinet (105 by 30 by 45 cm) with two adjustable shelves which could be positioned at

desired distances from the UV lamp. The top shelf had two holes (5.3 cm in diameter) which allowed irradiation of two "target spots" on the bottom shelf. By adjusting the top shelf to 5.7 cm and the bottom shelf to 23.5 cm from the lower surface of the UV bulb, a constant UV intensity of 100 $\mu W/cm^2$ (i.e., a 10-erg/mm^2 incident UV dose) at each target spot was maintained. The radiation intensity for each experiment was measured with a Blak-Ray J-225 UV photometer (Ultra-Violet Products, Inc., San Gabriel, Calif.). This instrument was calibrated and certified by Ultra-Violet Products with traceability to a National Bureau of Standards transfer meter (Eppley Laboratory, New Port, R.I.).

UV doses. Duplicate sets of 2- or 15-ml amounts of a suspension were dispensed into polyethylene bottles which were 65 mm deep and had a 10-mm opening at the top (S/R Natural Drop-Tainers, Alathon 20 low-density polyethylene; Wheaton Industries, Millville, N.J.) and irradiated simultaneously with incremental doses of UV. The depths of 2- and 15-ml suspensions were 6 and 40 mm, respectively. Two unexposed suspensions were included and served as controls in each study set. Immediately after exposure, 10-fold serial dilutions were prepared from each set. One-milliliter amounts from appropriate dilutions were dispensed into each of two petri plates (100 by 15 mm) to which 25-ml amounts of Trypticase soy agar were added. All plates were incubated at 32 to 35°C for 24 to 48 h, and then the colony-forming units were counted.

Regression analysis. Surviving fractions of initial populations were calculated from plate count data and plotted against doses of UV radiation. Dose-survivor data for each test set were analyzed by a Digital PDP-8 computer (Digital Equipment Corp., Maynard, Mass.) program, PLOTD, to determine the decimal reduction times, or D_{uv} values, and regression curves at a UV intensity of 100 $\mu W/cm^2$. Terms concerning UV radiation used in this paper have been defined previously (1).

RESULTS

The results of the inactivation experiments for the 10 strains of *P. aeruginosa* are summarized in Table 1. D_{uv} values ranged from 0.5 to 1.2 min, and corresponding correlation coefficients ranged from 0.951 to 0.998. Survivors from initial cell populations of 10^6/ml were recovered in only 1 of 40 tests after a radiation dose of 2,700 ergs/mm^2. Viable organisms were not recovered from any test suspension subsequent to exposure to a total UV dose of 3,000 ergs/mm^2.

Regression analysis of dose-survivor studies involving *P. aeruginosa* ATCC 9027 cell densities of 10^7/ml and 10^8/ml yielded D_{uv} values of 39.6 and 81.7 s, respectively (Fig. 1). Total kill of 10^7 cells per ml occurred between 210 and 240 s, whereas a 330-s exposure time (a 3,330-erg/mm^2 dose) reduced a 10^8-cell-per-ml suspension by 4 logs (a 99.99% kill). Doses greater than 3,300 ergs/mm^2 were not included in these studies.

UV resistance data, with correlation coefficients for each set of data, for additional microorganisms are listed in Table 2. *M. radiodurans* ATCC 13939, one of the more resistant organisms to ionizing radiations, was the most resistant organism included in this study and required a UV dose of 19,950 ergs/mm^2 (33.25-min exposure) to obtain a 90% kill. *C. albicans* ATCC 10231 was less resistant than *M. radiodurans*, but more resistant than any of the remaining organisms tested. A 7-min exposure was necessary for this *C. albicans* strain to absorb sufficient UV radiation to cause a 1-log reduction. The D_{uv} for *S. faecalis* ATCC 10541 was about one-third greater than that for *E. coli* ATCC 8739 and twice that calculated for *S. aureus* ATCC 6538. *P. diminuta* ATCC 11568 was the most resistant pseudomonad tested, and its dose-survivor profile was similar to that of *S. faecalis*.

TABLE 1. *Summary of D_{uv} values of 10 strains of P. aeruginosa exposed in polyethylene bottles*

P. aeruginosa strain	D_{uv} (s)[a]			
	Test 1	Test 2	Test 3	Test 4
ATCC 9027	37.5 (0.992)	34.2 (0.994)	43.7 (0.993)	38.5 (0.979)
ATCC 10145	43.9 (0.982)	47.1 (0.983)	45.7 (0.997)	48.5 (0.995)
ATCC 14207	25.7 (0.988)	26.9 (0.995)	42.9 (0.983)	50.7 (0.991)
ATCC 15442	31.4 (0.985)	34.9 (0.996)	41.9 (0.996)	43.1 (0.996)
ATCC 27853	57.9 (0.989)	54.6 (0.951)	42.2 (0.998)	39.9 (0.991)
B-2	69.1 (0.995)	55.8 (0.989)	51.6 (0.998)	46.8 (0.998)
G-2	29.1 (0.981)	32.1 (0.991)	28.2 (0.995)	29.1 (0.994)
BS-4	30.4 (0.993)	28.7 (0.996)	38.7 (0.995)	42.9 (0.994)
WB-1	72.0 (0.997)	56.4 (0.977)	51.4 (0.973)	54.0 (0.976)
SH-2918	33.4 (0.982)	32.8 (0.998)	33.2 (0.985)	42.5 (0.995)

[a] Length of time (seconds) at a UV intensity of 100 $\mu W/cm^2$ required to reduce the initial cell population (10^6 cells per ml) by 1 log (90%). Each value represents the average of duplicate sets of suspensions exposed at each time interval included in the study; numbers in parentheses are the correlation coefficients for each set of data.

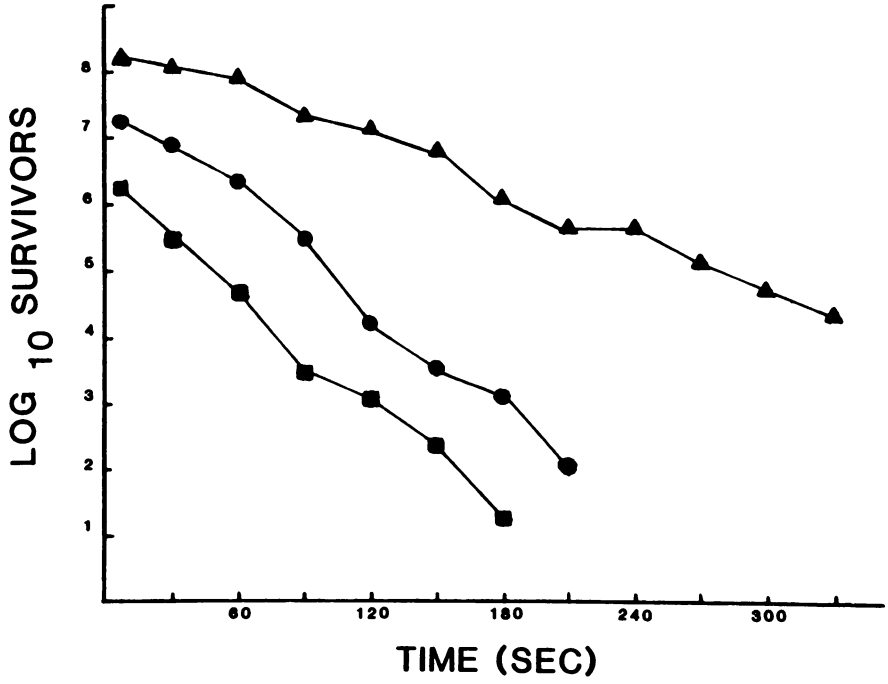

FIG. 1. *Comparisons of dose-survivor curves for 10^6/ml (■), 10^7/ml (●), and 10^8/ml (▲) saline suspensions of P. aeruginosa cells exposed to a UV intensity of 100 μW/cm² for various periods of time.*

D_{uv} values for 10^6- and 10^7-cell-per-ml suspensions of *P. aeruginosa* ATCC 9027, *M. radiodurans* ATCC 13939, *C. albicans* ATCC 10231, and *S. faecalis* ATCC 10541 exposed in 15-ml quantities (40-mm depth) were equivalent to the D_{uv} values obtained for these organisms after exposure in 2-ml amounts (6-mm depth).

Photoreactivation experiments were included in preliminary tests. Significant differences were not observed in the numbers of survivors recovered after subjecting irradiated suspensions to visible light or storing them in the dark. Test results indicated that these particular strains were not photoreactivated by the methods used.

DISCUSSION

The UV inactivation characteristics for many microorganisms have been reported (1, 7, 16, 21, 22), but sufficient and pertinent resistance data for a variety of *P. aeruginosa* strains were unavailable. Consequently, we designed a UV system so that we could determine the effectiveness of low-intensity UV energy for killing high levels of *P. aeruginosa*.

Zelle and Hollaender (22) reported that a UV dose of 550 ergs/mm² inhibited the formation of *P. aeruginosa* colonies by 90%. Antopol and Ellner (2) listed data that showed *P. aeruginosa* to be six times more resistant than *Legionella pneumophila* to UV radiation. Rubbo and Gardner (16) determined that certain gram-negative bacilli required 300- to 400-erg/mm² doses of UV to reduce initial populations by 1 log, but *P. aeruginosa* was not included in their tests. Our data showed that 270- to 720-erg/mm² doses were necessary for 90% inactivation of initial populations of the 10 *P. aeruginosa* strains tested.

Cells of *P. aeruginosa* were inactivated as readily in 15-ml suspensions (40-mm depth) as in 2-ml quantities (6-mm depth). These data demonstrated that a UV intensity of only 100 μW/cm² penetrated sufficiently through polyethylene bottles containing saline suspensions to obtain a total kill of 10^6 or 10^7 cells per ml in very short periods of time. Suspensions of 10^6 or 10^7 cells per ml yielded very similar D_{uv} values; however, suspensions of 10^8 cells per ml gave D_{uv} values that were twice as long. This finding is contrary to results reported by Collins (7), which showed that a 4-log reduction (99.99% kill) of 10^9 cells of *P. aeruginosa* NCTC 6750 occurred after inocula were exposed for 90 s. Perhaps this strain is sensitive, like most of ours, or the differences in results could be attributed to the methods used in exposing the organisms. Additional study is required to resolve this disagreement with other reports. Crowding or shadowing probably

TABLE 2. *Resistance data on selected organisms exposed to low-intensity UV radiation*[a]

Organism	D_{uv} (s)[b]		
	Test 1	Test 2	Test 3
M. radiodurans ATCC 13939	1995.0 (0.974)	1979.5 (0.988)	1983.7 (0.981)
S. aureus ATCC 6538	53.7 (0.988)	58.1 (0.986)	55.4 (0.986)
S. faecalis ATCC 10541	120.5 (0.988)	118.2 (0.986)	121.3 (0.988)
C. albicans ATCC 10231	438.5 (0.993)	450.5 (0.995)	452.8 (0.989)
E. coli ATCC 8739	81.6 (0.983)	82.8 (0.989)	79.5 (0.998)
P. diminuta ATCC 19146	73.8 (0.984)	76.3 (0.981)	72.1 (0.983)
P. diminuta ATCC 11568	116.3 (0.992)	121.2 (0.990)	118.4 (0.993)
P. maltophilia ATCC 13637	68.4 (0.978)	70.3 (0.981)	71.4 (0.985)
P. cepacia ATCC 25416	57.4 (0.988)	57.0 (0.982)	60.1 (0.987)
P. putrefaciens ATCC 8071	88.8 (0.990)	84.5 (0.987)	86.3 (0.988)

[a] UV radiation at an intensity of 100 $\mu W/cm^2$ was delivered to the cell suspensions; the distance from the lamp to the suspensions was 23.5 cm. Initial populations of 10^6 cells per ml were exposed in 2-ml volumes of saline contained in open polyethylene bottles.

[b] Time (seconds) required, at a UV intensity of 100 $\mu W/cm^2$, to reduce the initial cell population by 1 log (90%); numbers in parentheses are the correlation coefficients for each set of data.

prevented some cells from being subjected to a lethal dose of UV. The survivor curves obtained gave straight-line responses, without the presence of an initial shoulder or a "tailing" effect.

Only one set of exposure-recovery parameters was investigated. UV-irradiated cells were (i) recovered by using only one medium (Trypticase soy agar), (ii) exposed and diluted in 0.85% saline, (iii) exposed to UV under ambient temperature and relative humidity, and (iv) recovered by the pour plate method, using an incubation temperature of 32 to 35°C. The use of other conditions, such as suboptimal incubation temperatures and different media, could have altered the sensitivity of the recovery procedure and affected the D_{uv} values to some extent. In an earlier study (1), a broth dilution–most-probable-number procedure did enhance the recovery of UV-irradiated *Bacillus pumilus* spores. Preliminary studies in this investigation with a most-probable-number method failed to offer any advantage over the agar plate method for recovering greater numbers of *P. aeruginosa* survivors after UV irradiation.

Although photoreactivation of UV-irradiated organisms by visible light has been demonstrated, we agree with other investigators (16, 22) that this phenomenon has no practical significance in UV sterilization.

Each method of sterilization has its limitations, and certainly UV is no exception; however, data from this study demonstrated that even a low-intensity UV radiation of 100 $\mu W/cm^2$ is a very efficient and feasible sterilant. Quartz lamps are now available that can deliver very high UV doses in very short periods of time. UV systems can be developed in which such high doses of UV radiation can be generated, allowing shorter exposure times with greater killing capabilities.

Although UV has some limitations as a sterilant, certain solutions and articles that cannot be subjected to conventional decontamination may lend themselves to sterilization by UV. Data presented in this paper show that *P. aeruginosa* is very sensitive to low-intensity UV radiation when compared with other microorganisms (1, 7, 16, 22). The system used in this study proved to be useful in destroying *P. aeruginosa* and other pathogenic organisms. Modification of this system may be applied in the sterilization of other articles.

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