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Photoreactivation of UV-Irradiated *Legionella pneumophila* and Other *Legionella* Species

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Shortwave UV light was assessed as a feasible modality for the control of Legionnaires disease bacterium in water. The results of this study show that *Legionella pneumophila* and six other *Legionella* species are very sensitive to low doses of UV. However, all *Legionella* species tested effectively countered the germicidal effect of UV when subsequently exposed to photoreactivating light.

Legionella pneumophila, the etiological agent of legionellosis, has several markedly different clinical presentations. The pneumonic form of legionellosis (Legionnaires disease) can occur as a common-source outbreak with clinical findings of fever and pneumonia associated with high fatality rates; in the 1976 outbreak in Philadelphia, Pa., 182 people became ill of 29 whom died (7, 17). The nonpneumonic form of legionellosis (Pontiac fever) can occur as a common-source outbreak with the absence of pneumonia and mortality, as typified by the 1968 outbreak in Pontiac, Mich. (18, 30). Hospital-acquired Legionnaires disease with high fatality rates is a persistent problem among seriously ill and immunocompromised patients (22, 27). Sporadic cases of Legionnaires disease also have occurred in previously healthy patients (3, 20). It has been estimated that there are as many as 25,000 undiagnosed cases of legionellosis in the United States per year (16); it may be the most common cause of atypical pneumonia. Legionnaires disease bacterium appears to be a common environmental bacterium, ubiquitous in nature. It has been recovered from soil and water sources in both epidemic and nonepidemic areas (15) and may be part of natural aquatic ecosystems (14, 38).

Irradiation of cells with UV light (220 to 320 nm) can result in the formation of intrastrand cyclobutyl-pyrimidine dimers in the DNA, leading to mutagenic changes or cell death. Several repair pathways exist for the repair of UV-induced DNA damage including photoreactivation, excision repair, recombinational repair, and inducible error-prone repair (28). Photoreactivation is the error-free, light-dependent (300 to 600 nm), enzymatic monomerization of UV-induced pyrimidine dimers. The photoreactivating enzyme has been found in many species of bacteria (26), as well as in animals (37) and plant cells (25).

Light-induced recovery from UV damage has not previously been reported in the family *Legionellaceae*. This report compares the sensitivities of *Legionella* species to the germicidal effects of UV light. The report also describes for the first time the repair of UV-damaged DNA by photoreactivation in *L. pneumophila* and in other *Legionella* species. The effects of these findings on the control of Legionnaires disease bacteria in hospital environments are discussed.

MATERIALS AND METHODS

Bacterial strains. *L. pneumophila* Philadelphia 1 (5), *Legionella bozemanii* WIGA (4), *Legionella dumoffii* TEX-KL (4), and *Legionella micdadei* TATLOCK (24) were obtained from the Centers for Disease Control, Atlanta, Ga. *Legionella jordanis* ATCC 33623 (8), *Legionella oakridgensis* ATCC 33761 (32), and *Legionella wadsworthii* ATCC 33877 (12) were obtained from the American Type Culture Collection, Rockville, Md. *Escherichia coli* B/r ATCC 12407 (41) was used as a reference organism for UV sensitivity. *E. coli* AB2480 (23), which is a *recA*$^-$ *uvrA*$^-$ mutant, was kindly supplied by B. J. Backmann, *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

Media. Charcoal-yeast extract (CYE) agar (13) was composed of 1.0% yeast extract (Difco Laboratories), 0.2% activated charcoal (Norit A), 0.04% L-cysteine hydrochloride (Sigma Chemical Co.), 0.025% ferric nitrate (Fisher Scientific Co.), and 1.7% agar (Difco) in distilled water. The basic medium was autoclaved and cooled to 50°C, filter-sterilized L-cysteine and ferric nitrate were added, and the medium was adjusted with sterile 1 N KOH to yield a final pH of 6.9 to 7.0.

Yeast extract broth (YEB) (34) was composed of 1.0% yeast extract, 0.04% L-cysteine hydrochloride, and 0.025% ferric pyrophosphate (Biological Products Div., Centers for Disease Control) in distilled water. The medium was sterilized by filtration through a 0.45-μm filter (Millipore Corp.) and then adjusted to a pH of 6.9. Tissue culture-clean glassware was used to avoid any trace of detergents in the media.

E. coli cultures were grown in nutrient broth (Difco) and on nutrient agar (Difco).

Microscopy. All strains were monitored for contamination by plating on blood agar and by microscopic examination. Slides were air dried, briefly heat fixed, and stained with Gram crystal violet and then with Lugol iodine (10).

Qualitative tests for photoreactivation. A 1-ml volume of 60-h-old stationary-phase culture of *L. pneumophila* in YEB was used to inoculate 500 ml of fresh YEB in a 1-liter Erlenmeyer flask. The culture was shaken in a New Brunswick incubator shaker at 37°C for 30 h. The mid-log-phase cells were centrifuged and resuspended in sterile distilled water to a Klett of 110. Confluent lawns were prepared on petri dishes (150 by 25 mm; Becton Dickinson Labware, Oxnard, Calif. [Div. Becton, Dickinson and Co.]) containing CYE agar by spreading 0.3 ml of the cell suspension per plate with a bent glass rod. A piece of cardboard was used to cover half of an open plate, which was then UV irradiated at an intensity of 7 W/m^2 (equivalent to 700 μW/cm^2 or 7 J/m^2 · s). Duplicate plates were prepared for each UV exposure. Half the plates were kept in the dark, whereas the other half were light exposed for 60 min with indirect sunlight. The plates were then incubated in the dark at 37°C for 4 to 5 days.

A mid-log-phase culture of *L. pneumophila* in YEB was grown as described above, centrifuged, and resuspended in sterile distilled water to a Klett of 110. A 10-fold serial dilution was prepared from 10^0 to 10^{-7}. A 10-μl sample from each dilution tube was spotted on the surface of a CYE agar plate. Duplicate plates were prepared for each UV exposure. One plate from each time point was exposed to indirect sunlight for 60 min, whereas the duplicate plate was kept in the dark. All plates were then incubated in the dark at 37°C for 4 to 5 days.

Quantitative tests for photoreactivation and relative UV sensitivity. Cells grown on CYE agar plates at 37°C for 4 to 5 days were harvested and diluted with sterile distilled water to a Klett of 110 (approximately 5×10^8 CFU/ml). Tenfold serial dilutions were prepared, and appropriate dilutions (0.1 ml) were plated on CYE agar. The open plates were UV irradiated for 0, 5, 10, 15, 20, 25, or 30 s with a General Electric 15-W G15T8 germicidal lamp (254 nm). The flux was 2.4 W/m^2 (equivalent to 240 μW/cm^2 or 2.4 J/m^2 · s) as measured with a shortwave UV meter (model No. J225; Ultra-Violet Products Inc.). Unwanted photoreactivation was avoided by working in a darkroom with an orange photographic safety light. Half the plates were kept in the dark, whereas the other half were light exposed for 60 min with two General Electric 15-W F15T8 black-light-blue fluorescent lamps. Indirect sunlight was also used as a photoreactivating light source. The plates were incubated in the

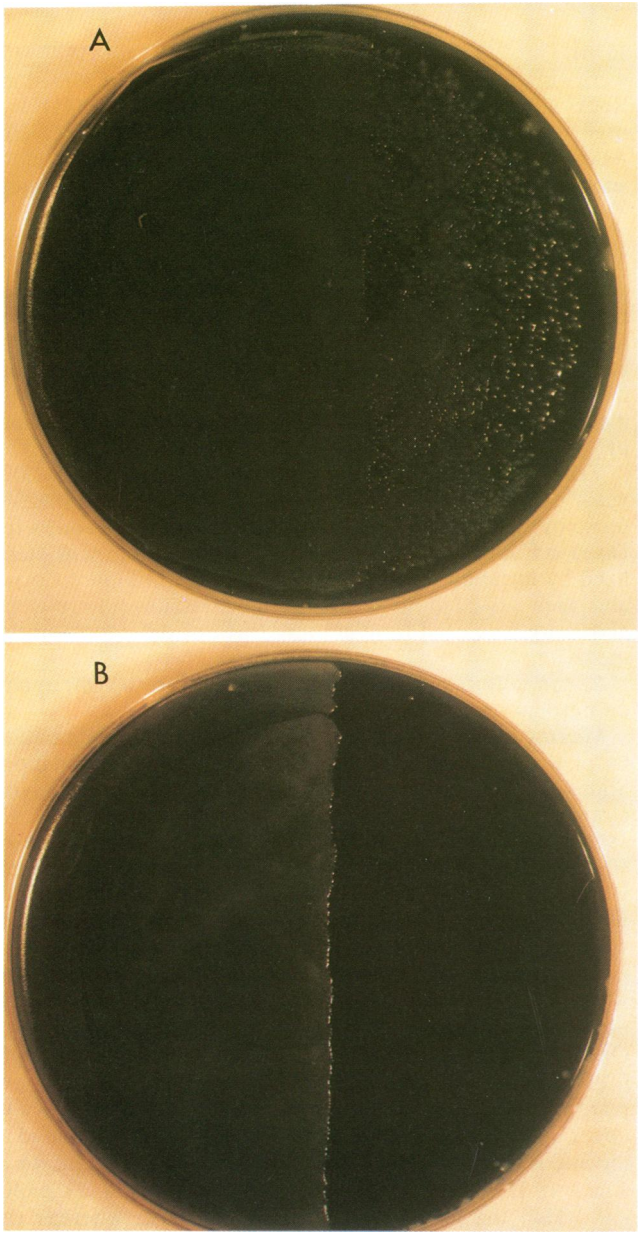

FIG. 1. Half of each plate, with a confluent lawn of *L. pneumophila*, was exposed to UV light. Plate A was then light exposed for 60 min, whereas plate B was kept in the dark.

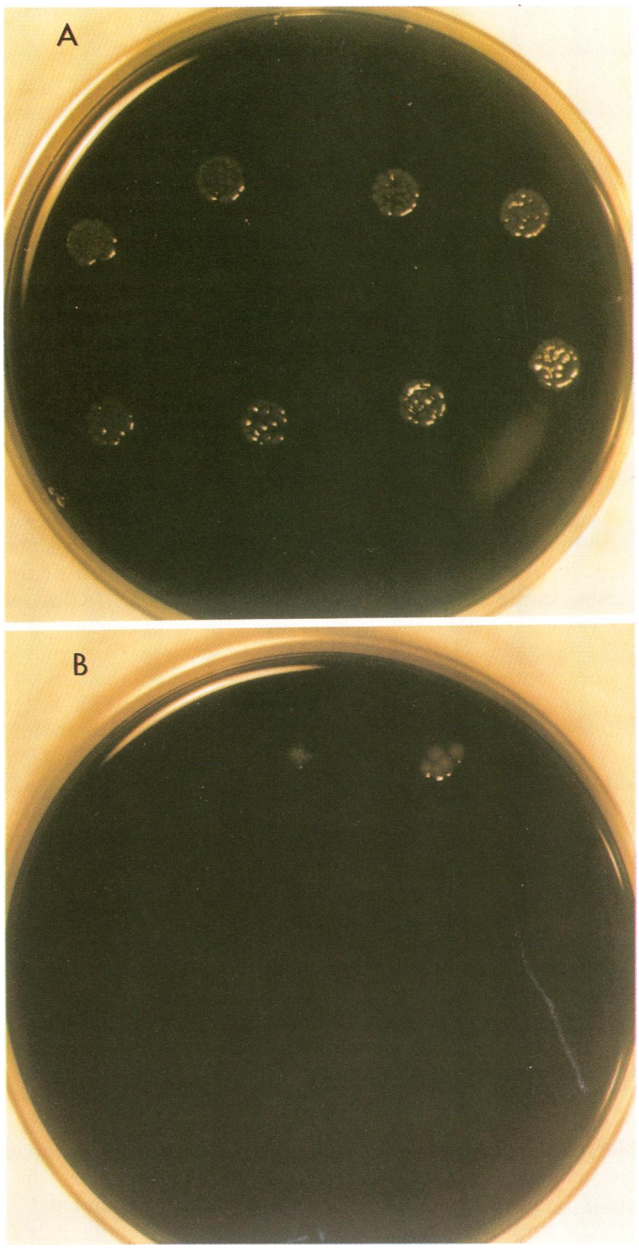

FIG. 2. Ten-microliter samples from 10^0 through 10^{-7} serial dilution tubes of *L. pneumophila* were spotted on duplicate CYE agar plates. Plate A was UV irradiated for 35 s and then light exposed for 60 min. Plate B was UV irradiated for only 15 s but was kept in the dark.

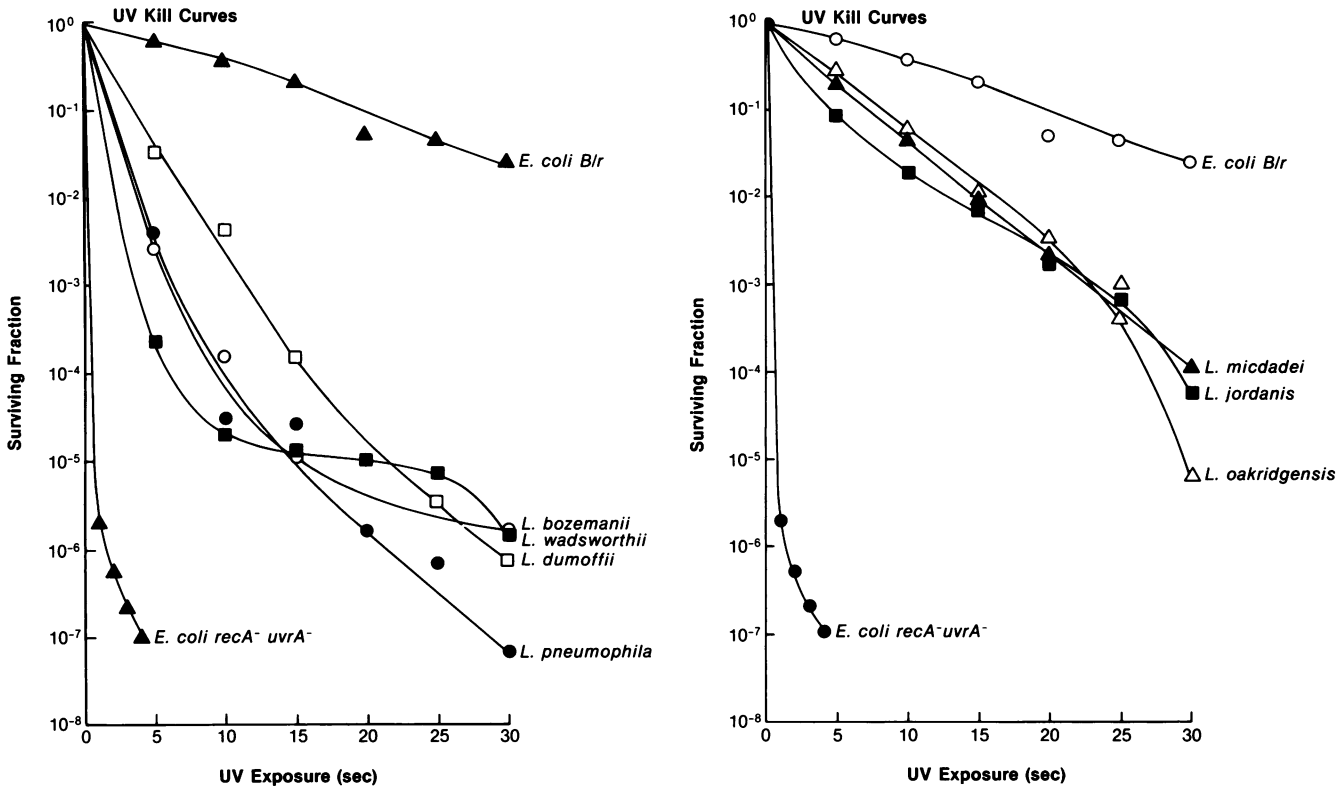

FIG. 3. Comparison of UV sensitivity among seven *Legionella* species and *E. coli* controls.

dark at 37°C for 4 to 5 days, colonies were counted, and the surviving fraction was plotted as a function of the UV dose.

RESULTS

Qualitative tests for photoreactivation. A rapid qualitative test for the presence or absence of photoreactivation in *Legionella* species was performed as described above. Figure 1 shows the result of a typical qualitative test for photoreactivation with *L. pneumophila*. The extreme sensitivity of *L. pneumophila* to UV irradiation is shown by the difference between the amount of growth on the left half of plate B, which was shielded from UV, and that on the right half of plate B, which was exposed to UV. Subsequent exposure to visible light resulted in substantial photoreactivation, as seen by comparing the right half of plate B, which was UV irradiated and incubated in the dark, with the right half of plate A, which received the same dose of UV but was then light exposed for 60 min.

Similarly, Figure 2 demonstrates a method for a rapid qualitative check for photoreactivation. Cells were serially diluted and spotted on CYE agar plates. Plate B (Fig. 2) was exposed to 15- s of UV irradiation and incubated in the dark. This resulted in the complete killing of all cells in each dilution spot except for the 10-μl spots of undiluted cells and cells diluted 1 to 10. In contrast, plate A, which had been UV irradiated for 35 s and then light exposed, showed growth on all dilution spots including the 10^{-7} dilution spot.

Quantitative tests for photoreactivation. There was as much as a 4-log increase in survival in UV-irradiated *E. coli* B/r cells exposed to photoreactivating light compared with cells grown in the absence of photoreactivating light. The differences in UV sensitivity due to dark repair are also evident from the UV kill curves of *E. coli* B/r and the *E. coli* mutant deficient in recombination and excision repair.

UV dose-survival curves of seven *Legionella* species kept in the dark after UV irradiation are compared (Fig. 3). The UV kill curves of *E. coli* B/r and *E. coli recA⁻ uvrA⁻* are included for references. *L. bozemanii*, *L. wadsworthii*, *L. dumoffii*, and *L. pneumophila* had similar UV kill curves and were more sensitive to the germicidal effects of UV irradiation than *L. micdadei*, *L. jordanis*, and *L. oakridgensis*. At UV exposures greater than 15 s, the rate of inactivation for *L. bozemanii* and *L. wadsworthii* decreased, suggesting the presence of a subpopulation of cells which are more resistant to the lethal effects of UV.

Results of experiments to determine the increase in survival resulting from photoreactivation of *L. pneumophila* and *L. dumoffii* are illustrated in Fig. 4 and 5, respectively. Other *Legionella* species tested showed similar results (Table 1).

DISCUSSION

Legionellosis appears to be transmitted by the airborne route and acquired by inhalation. Colonization of air-conditioning cooling towers by Legionnaires disease bacterium is potentially a major public health problem (11). This is of particular concern at health-care facilities with patients receiving immunosuppressive therapy and patients with serious underlying illness since these patients are at higher risk for nosocomially acquired Legionnaires disease pneumonia (21). There have been reports of *L. pneumophila* isolated from potable and nonpotable hospital water supplies (9, 39) and of *L. micdadei* isolated from nebulizers from respiratory therapy equipment (19).

The experiments reported here demonstrate that *L. pneumophila* and six other phenotypically related but genetically distinct *Legionella* species are sensitive to low doses of shortwave UV light. The sensitivity of *L. pneumophila* to

UV radiation suggests that mercury vapor germicidal lights might be useful in the management of Legionnaires disease by the UV irradiation of water and air within ventilating systems (1). However, the data presented here demonstrate that *L. pneumophila* has a very effective light-dependent DNA repair system. Each of the other six *Legionella* species tested also demonstrated effective photoreactivation. In some trials the germicidal effect of the UV light was countered by as much as a 4-log increase in survival when the UV-irradiated cells were exposed to photoreactivating light.

Members of the family *Legionellaceae* produce a soluble brown melaninlike pigment when grown on medium containing L-tyrosine (2). The pigment may affect UV sensitivity, although studies with other pigment-producing bacteria have indicated that pigment production is generally not effective as a protection against UV light (31).

Carson and Petersen (6) have reported that by using a commercial UV water sterilizer unit they could sterilize water, in the dark, containing levels of 10^5 to 10^6 cells of *Pseudomonas cepacia* per ml. However, if the water was exposed to average room light conditions after UV exposure, there was significant photoreactivation and subsequent growth of the organism. This reaffirms the need for caution in assessing the effectiveness of UV irradiation as a means of eliminating waterborne gram-negative organisms in hospital water supplies, particularly where the UV-treated water will be exposed to visible light during storage before use.

Although *L. pneumophila* can be rapidly inactivated at free chlorine concentrations of 3.3 and 6.6 mg/liter (35), the levels of residual chlorine which are found in public water distribution systems are not effective in the elimination of *L. pneumophila* when present in high numbers (29). Stout and colleagues (36) have suggested that *L. pneumophila* could be introduced into hospital potable water supplies from public water reservoirs. The organisms could then proliferate in the hot-water system and be disseminated to patient areas through the hospital water distribution system (40). It has recently been suggested that the most effective way to reduce the *L. pneumophila* cell count, and the incidence of nosocomial Legionnaires disease, would be to raise the

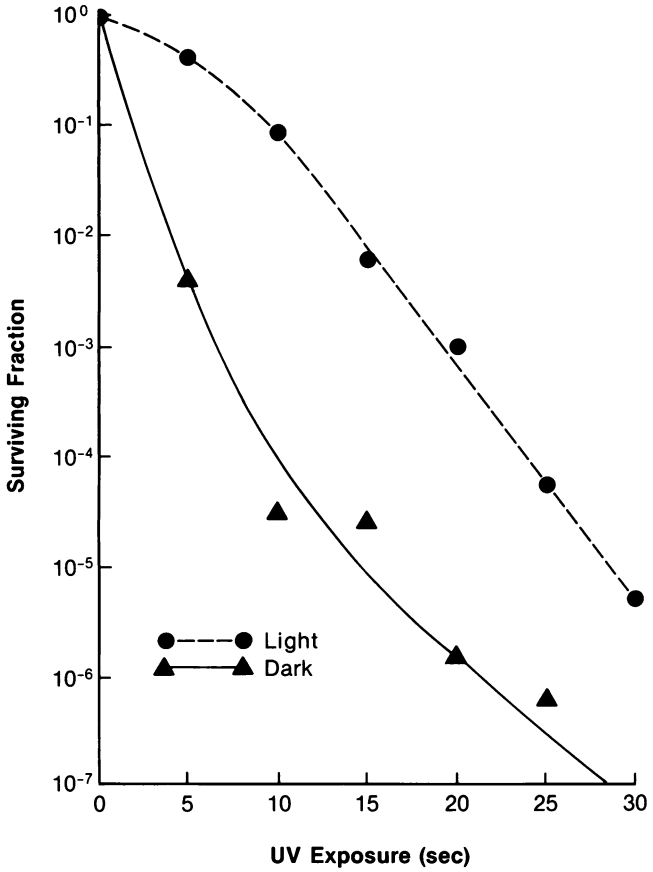

FIG. 4. UV dose-survival curves of *L. pneumophila* (L1) with photoreactivating light (●) and in the dark (▲).

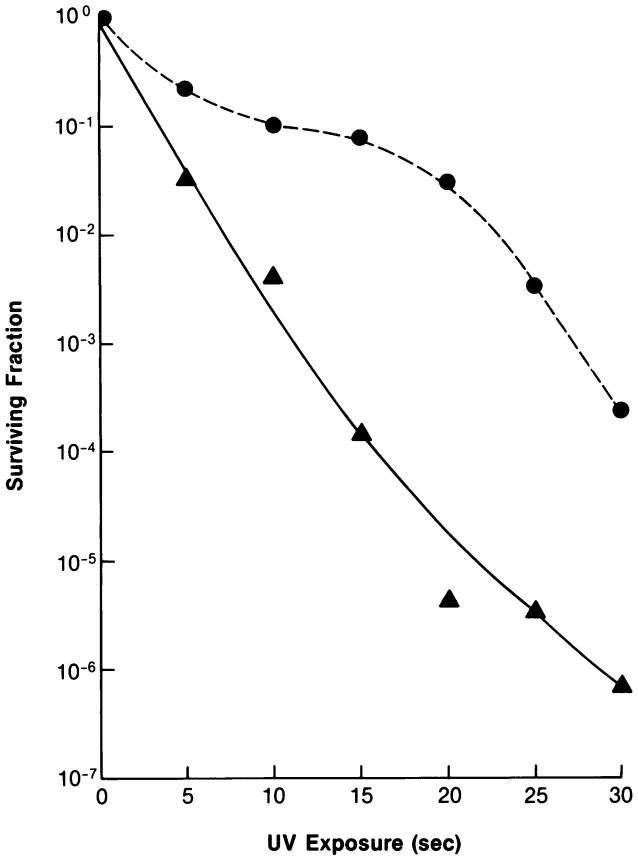

FIG. 5. UV dose-survival curves of *L. dumoffii* (TEX-KL) with photoreactivating light (●) and in the dark (▲).

TABLE 1. UV irradiation required to kill 90, 99, and 99.9% of the cells in the dark and with reactivating light

Species	UV irradiation (J/m^2) to kill:					
	90%		99%		99.9%	
	Dark	Light	Dark	Light	Dark	Light
E. coli B/r	53	110	84	223	103	ND[a]
L. oakridgensis	22	34	40	50	55	62
L. micdadei	18	38	36	62	54	ND
L. jordanis	11	30	31	72	55	ND
L. dumoffii	8	24	18	53	28	65
L. pneumophila	5	23	10	35	16	46
L. bozemanii	4	5	8	11	15	19
L. wadsworthii	4	19	6	34	8	50
E. coli recA⁻ uvrA⁻	0.2	0.5	0.7	1	1	2

[a] ND, Not determined.

temperature of hospital central water supplies every 2 months to 77°C for 72 h and then to flush the hot water systems (33). However, effective measures for the prevention and control of Legionnaires disease will require a more thorough understanding of the mode of transmission, mechanisms of aerosolization, and factors influencing the virulence of this intriguing bacterium.

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