

Photoreactivation in Airborne *Mycobacterium parafortuitum*

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Photoreactivation was observed in airborne *Mycobacterium parafortuitum* exposed concurrently to UV radiation (254 nm) and visible light. Photoreactivation rates of airborne cells increased with increasing relative humidity (RH) and decreased with increasing UV dose. Under a constant UV dose with visible light absent, the UV inactivation rate of airborne *M. parafortuitum* cells decreased by a factor of 4 as RH increased from 40 to 95%; however, under identical conditions with visible light present, the UV inactivation rate of airborne cells decreased only by a factor of 2. When irradiated in the absence of visible light, cellular cyclobutane thymine dimer content of UV-irradiated airborne *M. parafortuitum* and *Serratia marcescens* increased in response to RH increases. Results suggest that, unlike in waterborne bacteria, cyclobutane thymine dimers are not the most significant form of UV-induced DNA damage incurred by airborne bacteria and that the distribution of DNA photoproducts incorporated into UV-irradiated airborne cells is a function of RH.

Indoor and outdoor air contains suspended biological particulate matter that can include viable, pathogenic bacteria and viruses. Airborne bacteria, such as *Mycobacterium tuberculosis*, can pose a threat to the general public health through infectious disease. Environments associated with correctional facilities (35), health care facilities (3, 24, 25), shelters for the homeless, wastewater treatment plants (6), public transit systems (27), and schools (44) have been implicated as high risk due to their bioaerosol exposure potential. The possibility for disease transfer associated with infectious bioaerosols has prompted efforts to design economical systems to remove, disinfect, or otherwise inactivate bioaerosols in indoor environments.

UV irradiation of indoor air has received heightened attention in recent years due to its potential as a supplementary engineering control to inactivate infectious bioaerosols (4). Above certain dose levels, UV irradiation has been shown to inactivate airborne bacteria, and limited experimental and anecdotal evidence suggests that UV lamps may be effective and economical tools against the spread of some infectious airborne diseases when strategically placed in high-risk indoor environments (24, 25, 46, 55, 61). Although the production and use of UV air systems are growing in the United States (19), little is known regarding the phenomena governing UV inactivation of bacteria suspended in air. Empirical observations have implicated relative humidity (RH) as a major environmental factor influencing the UV-induced inactivation rates of airborne bacteria (37, 45); however, the fundamental mechanism(s) governing the response of airborne bacteria to UV irradiation at different RH levels has yet to be confirmed. Also not confirmed is the in situ recovery potential of UV-damaged airborne bacteria facilitated by light-activated DNA photolyase enzymes that repair cyclobutane thymine dimer DNA lesions (i.e., photoreactivation [PR]).

Room fluorescent and incandescent light and sunlight are often present together with UV light in both natural settings and engineered UV aerosol disinfection systems. The well-documented magnitudes of PR observed in UV-inactivated waterborne bacteria suggest that PR may adversely influence the performance of UV bioaerosol irradiation systems. PR studies of bacteria suspended in liquid or distributed on agar plate surfaces have empirically identified the degree of UV-induced damage (22) and the length of exposure to photoreactivating light (8, 21) as factors that affect the extent of PR. An inverse relationship between UV dose and the extent of PR has been observed for wastewater coliforms (22) and the bacterium *Haemophilus influenzae* (52). Temperature (8), visible light intensity, and wavelength (48) affect the photolyase-catalyzed DNA repair rate of thymine dimer lesions. PR reactions have been successfully modeled using first-order saturation-type kinetics (8). DNA photolyases have been found in members belonging to all three domains of life, but photolyase activity is absent from many genera in a seemingly unpredictable manner (48). Distinct pure bacterial cultures can have markedly different PR responses depending on the presence and type of DNA photolyase that they contain (48). The following classes of photoreactivating enzymes that require visible light to support their activity have been recognized: (i) a folate class enzyme, with a maximum activity near 380 nm, and (ii) a deazaflavin class enzyme, with a maximum activity near 440 nm (33, 48). Little is known regarding the induction of DNA photolyase production in bacteria. The induction system is more clearly understood in eukaryotes, for which evidence suggests that the transcription of the gene *PHR1* that encodes the apoenzyme for DNA photolyase in the yeast *Saccharomyces cerevisiae* is induced in response to 254-nm radiation (50).

PR investigations performed in liquid suspension or on agar surfaces may not accurately reflect airborne PR behavior because the hydration states experienced by airborne bacteria are much different than those in aquatic environments or under culturing conditions. Airborne bacteria and spores exist in a partially hydrated state that depends on their physiology and the ambient RH level. The degree of hydration may change

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intracellular DNA and protein conformations (9) and thus may affect the type of DNA damage that an airborne UV-irradiated organism experiences, as well as any potential recovery facilitated by photolyase enzymes.

Previous laboratory-scale investigations have yielded information regarding the PR potential of some important airborne pathogens in liquid and on agar, but there have been no investigations reporting the in situ PR potential of airborne bacteria. PR ability has been observed in many *Mycobacterium* species, including *M. smegmatis* (11) and *M. tuberculosis* H37Ra (7). Photolyase activity is apparently absent in *Mycobacterium phlei* (11) and *Bacillus subtilis* spores and vegetative cells (33). In a previous experiment aimed at demonstrating the photoreactivating potential of airborne bacteria, *M. smegmatis* bioaerosols challenged exclusively with UV radiation showed a limited ability to photoreactivate (less than 10% increase in culturability) when illuminated with visible light on agar surfaces following their collection from air (11).

Due to the presence of sunlight, fluorescent light, and incandescent light in indoor areas where UV lamps are strategically placed to inactivate infectious bioaerosols, there is a need to determine if PR indeed occurs within airborne bacteria and, if so, to quantify these PR rates. To date, bacterial PR studies extrapolated to aerosol environments have been limited to post-aerosol collection on agar surfaces following exposure to UV radiation. We report here in situ observations of PR occurring within bacteria while airborne at multiple RH levels and UV doses. Cyclobutane thymine dimers were measured in airborne bacteria at low and high RH levels, and the results were used to suggest a fundamental mechanism for the RH dependence of UV inactivation and PR in bacterial bioaerosols.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. *Mycobacterium parafortuitum* (ATCC 19689) is a rod that is 2 to 4 μm in length, yields pale yellow colonies, has a G+C content of 62 to 70 mol%, and is acid fast (58, 60). *Serratia marcescens* (ATCC 13880) is a gram-negative rod with a G+C content of 53 to 59 mol% (12). *Bacillus subtilis* spores (ATCC 90287) were isolated from vegetative cells, are 1.5 to 1.8 μm in length, and have a G+C content of 42 to 43 mol% (5). *M. parafortuitum* and *S. marcescens* were grown at 37°C on soybean-casein digest agar (SCDA) (Difco Laboratories, Detroit, Mich.). *S. marcescens* was incubated for 24 h, while *M. parafortuitum* was incubated for 60 h. Sporulating *B. subtilis* cultures were grown on agar plates (8.5 g of nutrient agar with 0.002% $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$) (47) per liter at 37°C. After 5 days, spores and cells were removed from the agar surface by scraping the plates with a sterile glass rod. Spores and cells were then suspended in sterile deionized water. Spores were separated from remaining vegetative cells by repeated centrifugation at 10,000 rpm at 22°C for 8 min and then decanting the top layer of vegetative cells (spores settle before vegetative cells) until the solution was greater than 99% free of vegetative cells. The ratio of spores to vegetative cells was determined by light microscopy using the Schaeffer-Fulton spore stain method (49). Spores were not pasteurized (heated at 80°C), as this step was found to increase the germination potential of spores prior to UV exposure and during impinger collection. Spores were stored at 4°C in sterile deionized water for a maximum of 24 h prior to their use.

Pure cultures of *B. subtilis* vegetative cells, *M. parafortuitum*, and *S. marcescens* were removed from agar plate surfaces by scraping and suspended in sterile deionized water or in 15 mM phosphate-buffered saline (PBS; 10 mM sodium phosphate buffer, 5 mM NaCl [pH 7.2]) prior to nebulization. Immediately before aerosolization, cell and spore solutions were diluted to a concentration of approximately $10^9/\text{ml}$ as determined by direct microscopy or absorbance at 660 nm (calibrated by direct microscopy). For experiments in which cells were irradiated in liquid suspension, bacteria were added to sterile distilled water to a final concentration of approximately $10^9/\text{ml}$. Microscopic examination of bacte-

rial suspensions (via wet mount) confirmed the dispersed state of cells prior to their aerosolization or use in liquid experiments.

Bacterial enumeration. Both culturable and total bacteria (culturable and nonculturable) were quantified from impinger-recovered air samples and liquid-batch UV experiments. A modification of the standard plate count method was used to enumerate culturable bacteria. Within 2 h after collection, samples from liquid impingers were diluted (usually 1:10) in 50 mM PBS (150 mM NaCl [pH 7.2]) solution and plated using a spiral dispensing method (Spiral Biotech, Inc. Bethesda, Md.) according to the manufacturer's recommendations. At least three replicates of each sample were plated. *S. marcescens* and *B. subtilis* spores were incubated at 37°C for 24 h, and *M. parafortuitum* was incubated at 37°C for 60 h. All organisms were cultured on SCDA agar. A comparison of spiral plate counts with standard spread plate counts showed no difference between the culturing recovery of these methods (based on an independent *t* test, $\alpha = 0.05$); the spiral plating method variability was lower than that of the spread plate method (coefficient of variance [CV] was 5% lower for the spiral plating method [$n = 10$]). All plating was performed in indirect, dimmed light, and incubations were completely protected from light to control for PR.

Epifluorescence microscopy was used to enumerate total bacteria. Vegetative cells and spores were stained with 4',6'-diamidino-2-phenylindole (DAPI) (Sigma Chemicals, St. Louis, Mo.), a DNA-binding fluorescent stain (59), in accordance with previously described methods (14). Samples for total counts were stained at a final concentration of 0.5 to 1.0 μg of DAPI per ml. Vegetative cells and spores were incubated in DAPI for 2 min at room temperature and then filtered through 25-mm-diameter, 0.2- μm (average pore size) black polycarbonate filters (Poretics, Inc., Livermore, Calif.) as previously described (15). Filters were mounted using low-fluorescence immersion oil and examined at a magnification of $\times 1,100$ using a Nikon Eclipse E400 epifluorescence microscope fitted with a mercury lamp and polarizing filters (HBO 100-W mercury lamp; D360/40 excitation filter; 420 emission filter; 400DCLP beamsplitter; Chroma Technology Corp., Brattleboro, Vt.). Between 5 and 10 random fields and more than 200 total cells were counted per slide. All direct counts were reported as the average for all fields counted. In accordance with the methods reported by Hernandez and coworkers (14), direct counts from aliquots having CVs greater than 30% were discarded, and new sample aliquots were stained and counted until a uniform distribution was observed.

Aerosol reactor. A bench-scale reactor was constructed to perform experiments that determine bacterial bioaerosol inactivation response from UV irradiation and UV irradiation-PR under sustained RH levels. The reactor was 0.8 m^3 , constructed of 1.27-cm-thick clear Lucite plastic, and operated in a completely mixed flowthrough mode. The reactor was completely mixed (ventilation effectiveness [ϵ_v] = 0.98 to 1.02, where $\epsilon_v = 1$ is completely mixed) by three 1.5-W fans (Cafra model 727TT; Wiarton, Ontario, Canada) and capable of sustaining air exchange rates between 0 and 7.3 h^{-1} , as determined by SF₆ tracer gas analysis. The environmental conditions within the reactor (temperature and RH) could be maintained at stable levels at any air exchange rate used.

Low-pressure, 30-W mercury vapor UV lamps (G30T8; Osram-Sylvania, Hanover, Mass.) were installed to irradiate the contents of the aerosol reactor. To provide a uniform line source, lamps were placed in each corner and extended the full height of the reactor. Lamps were wrapped in eight layers of aluminum filter mesh (Research Products Corporation, Madison, Wis.) to attain the desired experimental UV radiance levels. The spectral power distribution (SPD) measured for the UV lamps with screens showed that 95% of power was emitted at 253.7 nm after 100 h of operation. UV lamp SPDs were determined between 240 and 400 nm using a scanning radiometer (model ISADHL; Jobin Yvon/Horiba Inc., Edison, N.J.). The UV doses maintained during this study were commensurate with those of other indoor air quality studies reported previously (23, 43). Chemical actinometry served to quantify UV irradiance according to previously described methods (39, 42). Prior to UV inactivation and PR experiments, actinometry measured the multidirectional irradiance incident upon a spherical object, or spherical irradiance, at 25 multiple locations in the reactor and at each RH level tested. A 0.6 M solution of KI with 0.1 M KIO₃ in 0.01 M borate buffer (pH 9.25) was loaded into 0.5-cm³ spherical quartz actinometry cells. This solution is transparent to wavelengths greater than 330 nm and opaque to wavelengths less than 290 nm. In this chemical actinometry, triiodide is formed in the presence of UV irradiation. Triiodide was measured spectrophotometrically at 352 nm with a UV 160U Shimadzu recording spectrophotometer (Shimadzu Scientific Instruments, Columbia, Md.). The dose was calculated by exposing actinometry cells to UV irradiation for 30 min, measuring the triiodide concentration, and calculating UV dose according to published calibration data. Photoreactivating light was supplied by a 40-W full-spectrum natural fluorescent lamp (F40T12SUN; Verilux, Inc., Stamford, Conn.) placed in the center of the reactor. This light has an SPD that resembles that of noon on an overcast day in

June at midlatitudes. A complete description of the reactor has been presented previously (37).

Experimental protocols. Airborne UV inactivation and PR experiments were performed in the aerosol reactor after UV lamps were warmed to achieve operating temperature. Lamps were turned off, and bacteria were aerosolized into the reactor for 5 min (see "Aerosolization and collection" below). During the first 3 min of aerosolization, reactor RH was adjusted to a predetermined level and maintained throughout the experiment. Once aerosolization of bacteria was complete and reactor RH was stable, ventilation was initiated at a 3.5 h⁻¹ air exchange rate, and a zero time sample was collected to determine the initial airborne bacterial concentration. The UV lamps were then turned on, and five sequential air samples were taken at predetermined time intervals. The time intervals were set so that concentrations could be measured effectively through a 95% UV-induced reduction in culturable bacteria. After the final sample was collected, the reactor air was evacuated at a rate of 8 h⁻¹ air changes for 30 min in the presence of UV radiation with filter-sterilized air, and samples were analyzed for total and culturable bacteria and for cyclobutane thymine dimers (CTDs) in a subset of experiments. Before all experiments and after the final samples were collected, the reactor air was evacuated at a rate of 8 h⁻¹ air changes for 30 min in the presence of UV radiation with filter-sterilized air; following these evacuations, airborne bacterial concentrations were below detectable limits, as judged by direct or culturable counts. Experiments were performed for the following reactor scenarios: (i) UV lamps on, with no photoreactivating light at multiple RH levels, (ii) UV lamps on, with photoreactivation light on at multiple RH levels, and (iii) visible light on and off at fixed RH level for multiple UV irradiances.

Liquid-based UV inactivation and PR rates were determined as follows. Bacteria were suspended in sterile deionized water in plastic petri dishes (50 ml, 115-mm diameter by 10 mm) at a known UV spherical irradiance level (7.5 μW/cm², as determined by actinometry), and initial total and culturable concentrations were determined prior to starting the UV lamps. Bacteria were UV irradiated in the dark, and samples were taken until an approximately 100-fold reduction in culturability was observed. The UV lamps were then turned off, and visible-light lamps were turned on. Samples were taken after 10, 20, 30, 45, 60, and 90 min of visible-light exposure. PR control experiments were executed in a similar manner except post-UV samples were taken in the dark. All samples were stored in the dark at 4°C for less than 2 h and analyzed for total and culturable bacteria as described previously.

Samples taken represented a time series of total and culturable cell concentrations taken during all bench-scale air and liquid experiments. First-order rate coefficients were used to estimate the exponential decay of cells within the reactor according to a completely mixed flow reactor model described previously (37). PR rates were calculated as the difference of the overall culturable bacteria decay rates in the presence and absence of visible light in otherwise identical UV inactivation experiments.

For airborne and liquid base experiments, first-order reaction rate coefficients were normalized by average spherical irradiance and reported as *Z* values (in square centimeters per microwatt per second) (43; T. W. Kethley, unpublished data):

$$Z = \frac{k}{I} \quad (1)$$

where *Z* is the *Z* value response, *k* is the first-order loss rate coefficient (per hour), and *I* is the average reactor spherical irradiance (microwatts per square centimeter).

To estimate variability and determine an average *Z* value response for liquid-suspended cultures and each aerosolized bacterial culture under the RH condition tested and all liquid experiments, all UV irradiation, PR, and natural decay experiments were executed in at least three independent trials. All airborne bacterial concentration data for each treatment scenario were log transformed (natural log base) and pooled, and the rate coefficients were estimated using the least-squares method for determining best data fit. The linest function in MS Excel Software (Microsoft Inc., Redmond, Wash.) was used to estimate the standard error for the reported rate coefficients. To assess the differences between rate coefficients estimated for different experimental treatments, a method of dummy variables was used (2, 18). Standard errors were propagated for *Z* value response calculations through accepted methods of random-error propagation by linear combinations and multiplicative expressions (28).

Aerosolization and collection. Aerosols were generated using an air-jet nebulizer (Collison Six-jet; BGI Inc., Waltham, Mass.) operated at 20 lb/in² (16). Air supplying the nebulizer was carbon filtered and desiccated by passage through a filter apparatus (model 3074; TSI, Inc., St. Paul, Minn.) and particle filtered by

a 0.2-μm particle filter (Bacterial Air Vent; Gelman Sciences, Port Washington, N.Y.) immediately before introduction into the nebulizer. The nebulizer was directly connected to the front of the reactor by a 1.58-cm Swagelok compression fitting (Denver Valve and Fittings Co., Denver, Colo.). One 1.5-W fan was directed toward the nebulizer effluent to ensure its immediate mixing upon entrance into the reactor.

Glass impingers (AGI-30; Ace Glass Inc., Vineland, N.J.) collected bacteria from reactor air in accordance with accepted methods (56, 57). Before each experiment, impingers were washed with deionized water, autoclaved, and wrapped with aluminum foil to shield the collected bacteria from UV radiation and visible light. Sterile PBS (30 ml; 50 mM sodium phosphate buffer, 150 mM NaCl [pH 7.2]) served as the impinger collection medium. In the bench-scale reactor, six impinger sampling ports, each linked to flow meters with control valves (model 32460-48; Cole-Parmer, Vernon Hills, Ill.), were connected to a rotary vane-type vacuum pump (model 0523-V4A-G180DX; Gast Inc., Benton Harbor, Mich.), which collected air from each impinger at 12.5 liters/min. Impinger air flow rates were calibrated before each experiment using a bubble generator (Gilliam Instrument Corp., Clearwater, Fla.).

Assays for CTDs. CTDs were quantified by a Western blot assay. A secondary stock of antibodies conjugated to fluorescent reporter molecules served to quantify the amounts of bound primary (specific for CTDs) monoclonal antibodies for Western blots.

DNA extraction, measurement, and denaturing. UV-irradiated and nonirradiated bacterial DNA was extracted from whole cells in impinger-collected air samples using a genomic DNA purification kit (Wizard; Promega Corp., Madison, Wis.). *S. marcescens* samples were lysed using the kit lysing agent. *M. parafortuitum* samples were lysed by treatment with proteinase K (200 μg/ml) (Sigma Chemical) and lysozyme (10 mg/ml) (Sigma Chemical) in a 10 mM Tris-1 mM EDTA (pH 7.5) buffer for 1 h according to the methods of Anderberg and coworkers (1). Enzyme-treated *M. parafortuitum* cells were then suspended in 1 ml of lysis solution provided with the Wizard kit and placed in 2-ml screw-top vials with 0.5 g of 100-μm sterile glass beads (Biospec Products, Inc., Bartlesville, Okla.). The vials were shaken using a mini-bead beater (Biospec Products, Inc.) for 5 min at 5,000 cycles per min. The vials were then incubated at 65°C for 1 h and subsequently shaken at 5,000 cycles per min for 3 min. DNA was then isolated from cell lysate according to the kit manufacturer's instructions. The kit yielded genomic DNA (average size greater than 50 kb) dissolved in a 10 mM Tris (pH 7.7)-1 mM EDTA solution. Extracted DNA was denatured by heating in boiling water for 10 min followed by immediate cooling on ice for 5 min according to previously described methods (10). Extracted DNA was quantified using a DAPI-based fluorimetric dye-binding assay for irradiated and nonirradiated DNA (17). This protocol could accurately measure DNA concentrations above 5 ng.

Immunoblotting. CTDs were quantified by a Western blotting technique modified from methods described previously for quantifying UV-irradiated mammalian cells (10). Denatured DNA was immobilized in triplicate aliquots on 0.45-μm nylon transfer membranes (Immobilin NY+; Millipore Corp., Bedford, Mass.) using a 72-well vacuum slot blotter (Minifold II; Schleicher and Schuell, Keene, N.H.) operated according to the blotter manufacturer's recommendations. Concentrations of blotted DNA ranged between 5 and 500 ng/ml, depending on the amount recovered from the air samples and on the UV dose. Membranes were then removed from the blotter, air dried, and baked at 75°C for 2 h.

Blotted membranes were rehydrated in PBST (10 mM sodium phosphate buffer, 150 mM NaCl, 0.05% Tween 20 [pH 7.5]) in 7-ml sterile glass vials. The PBST was decanted, and the blotted membranes were incubated for 10 to 14 h in 2 ml of anti-CTD mouse-derived monoclonal antibodies (Kamiya Biomedical Company, Seattle, Wash.) (29) at an optimized 1:5,000 (wt/vol) dilution in PBST, including 4% membrane blocking agent (Amersham Pharmacia Biotech, Piscataway, N.J.). Incubation was performed in the dark at room temperature (22°C) on an orbital shaker at 150 rpm. After the primary incubation, the membrane was rinsed twice with 5 ml of PBST, washed in PBST for 15 min on an orbital shaker at 150 rpm, and rinsed again in 5 ml of PBST. This rinse-wash protocol was used for subsequent hybridization with secondary antibody stocks.

Bound primary antibody was quantified using a fluorescently conjugated anti-mouse immunoglobulin G (IgG) antibody. A 2-ml solution of goat-derived anti-mouse IgG whole-molecule antibody conjugated to Alexa fluor 488 dye (Molecular Probes, Eugene, Oreg.) was incubated with the membrane-bound DNA-primary antibody complex(es) at an optimized dilution of 1:10,000 (vol/vol) secondary antibody in PBST. Incubation continued for 3 h at room temperature in the dark on an orbital shaker at 150 rpm. Membrane blots were washed as previously described and air dried, and fluorescent signal was quantified on a Storm gel and blot imaging system (Molecular Dynamics, Sunnyvale, Calif.) using ImageQuant software (Molecular Dynamics) with a photomultiplier tube

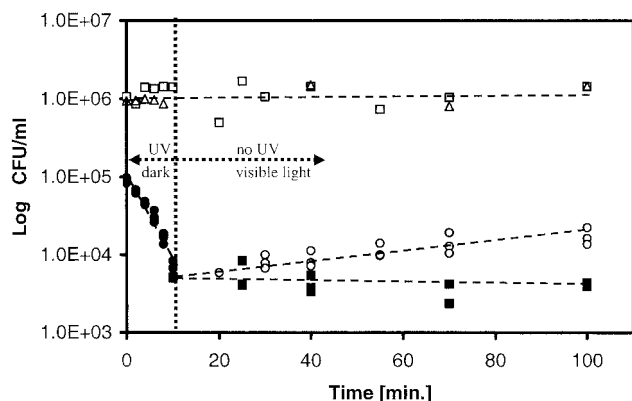


FIG. 1. UV-induced inactivation and PR of *M. parafortuitum* in liquid suspension. Results from three independent trials are presented. Symbols represent UV-irradiated total bacteria with visible light (\square) and without visible light (\triangle), culturable bacteria with visible light (\circ) and without visible light (\blacksquare), and culturable bacteria irradiated by UV (\bullet). Average UV spherical irradiance was 7.5 ± 0.3 (SE) $\mu\text{W}/\text{cm}^2$.

voltage of 800 and a blue chemifluorescence scanner setting. Quantification was based on measured volume (integrated intensity of all pixels in a defined area) using a local average background correction. The volume is directly proportional to the quantity of thymine dimer bound on the membrane.

This Western blot protocol was optimized for chemifluorescence quantification by independently varying primary and secondary antibody dilutions exposed to known quantities of damaged DNA until a linear response range of the fluorescent dye was obtained. The signal had a linear range of approximately 10-fold ($r^2 = 0.98$ for pooled results of three experiments), and this range extended to quantification below 2 ng of DNA (from a liquid suspension dose of $3,360 \mu\text{W} \cdot \text{s}/\text{cm}^2$ for *S. marcescens*).

RESULTS

PR in liquid suspension. The results of *M. parafortuitum* liquid PR experiments are summarized in Fig. 1. Following liquid UV irradiation that caused a greater than 90% culturability decrease, culturable *M. parafortuitum* concentrations increased markedly with time when incubated in the presence of visible light and decreased when incubated for the same time period in the dark. The normalized UV inactivation rate (*Z* value, dark) was $8.0 \times 10^{-4} \pm 0.5 \times 10^{-4}$ (standard error [SE]) $\text{cm}^2/\mu\text{W} \cdot \text{s}$. The PR rates subsequent to UV inactivation averaged 0.29 ± 0.1 (SE) h^{-1} . Total bacterial counts performed during UV irradiation and subsequent reactivating experiments did not change with time, confirming that increases or decreases measured in culturable cell concentrations were due only to UV irradiation damage, natural decay, and PR. Independent *t* tests ($\alpha = 0.05$) comparing the culturable rate responses in the presence and absence of visible light support the conclusion that *M. parafortuitum* is capable of repairing genomic UV damage (CTDs) via PR.

Airborne PR and RH. PR occurring within airborne bacteria was confirmed by concurrent exposure of airborne bacteria to UV irradiation and visible light confined within the bench-scale reactor. The PR rate in airborne *M. parafortuitum* was measured as the difference in *Z* value determined in the presence and absence of visible light and responded to changes in RH. Differences in *Z* value response were observed between experiments performed in the presence and absence of visible light but only when RH was greater than 80% (Fig. 2). *Z* value

TABLE 1. Decrease in *Z* value in the presence of PR light for airborne *M. parafortuitum* at different UV spherical irradiance levels^a

UV source (no. of lamps)	<i>Z</i> value response ($10^4 \text{ cm}^2/\mu\text{W} \cdot \text{s}$) (SE)	% PR (SE)	UV spherical irradiance ($\mu\text{W}/\text{cm}^2$) (SE)
1	3.7 (1.5)	43.6 (19.5)	1.88
2	6.6 (2.2)	65.5 (22.8)	3.7
3	2.82 (0.9)	37.0 (12.7)	5.6
4	0.5 (0.7)	6.9 (9.3)	7.5 (0.01)

^a All rates were measured at 95% RH. PR rates are defined as the difference between normalized UV inactivation rates in the presence and absence of visible light. Errors reported are 1 standard error. The differences in UV inactivation rates in the presence and absence of visible light were significant for all spherical irradiances except $7.5 \mu\text{W}/\text{cm}^2$ ($\alpha = 0.5$). % PR = $(Z_{\text{PR}}/Z_{\text{no PR}}) \times 100$.

responses were significantly lower when visible light was present ($\alpha = 0.05$) in tests where reactor atmosphere was maintained at 80 and 95% RH. A decrease in the *Z* value response of a factor of 2 (no visible light) was observed when RH was increased from 40 to 95%, and a decrease of a factor of 4 was observed for the same RH range when visible light was present.

Effect of UV dose on airborne PR rate. The effects of UV dose on the inactivation and PR of airborne bacteria were tested. UV inactivation experiments were conducted in the bench-scale reactor under conditions identical to the RH response experiments except the UV spherical irradiance was varied in the range between 1.9 and $7.5 \mu\text{W}/\text{cm}^2$ and RH was fixed at 95%. Figure 3 depicts the dose response of effective UV inactivation rates at 95% RH. In the absence of visible light, the response was a linear function of the UV spherical irradiance and confirmed the applicability of the equivalence of time and intensity (i.e., dose) in the inactivation of airborne bacteria at high RH levels. During experiments performed at 95% RH in the presence of visible light, PR rates were at a maximum when the reactor average UV irradiance was $3.7 \mu\text{W}/\text{cm}^2$, then decreased in response to increasing UV spherical irradiance until no PR was observed at $7.53 \mu\text{W}/\text{cm}^2$. Table 1 summarizes PR rates for the range of UV doses used.

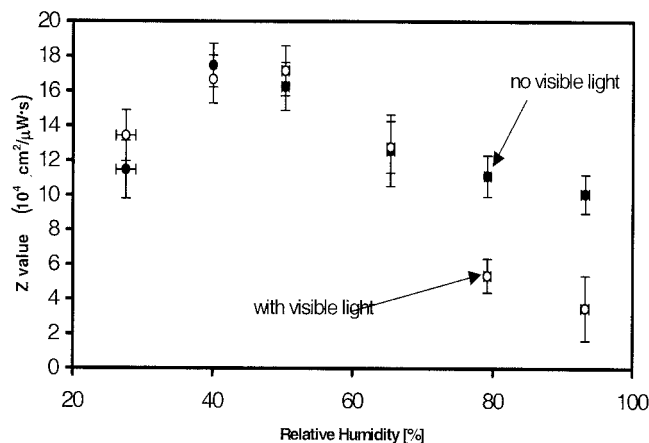


FIG. 2. *Z* value response for airborne *M. parafortuitum* in the presence (\circ) and absence (\bullet) of visible light. UV spherical irradiance was $3.7 \mu\text{W}/\text{cm}^2$. All error bars represent 1 SE.

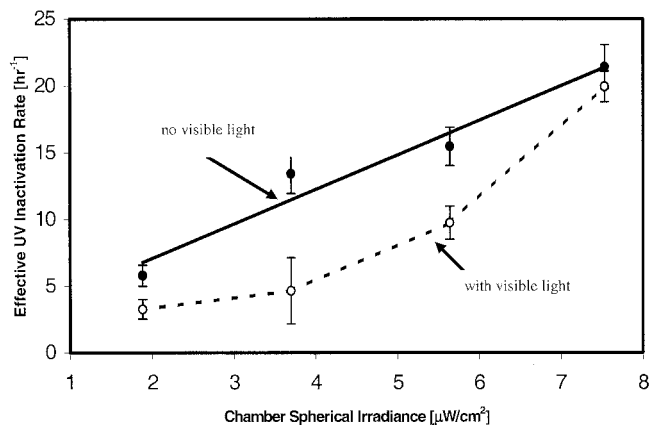


FIG. 3. UV inactivation rate for *M. parafortuitum* at different UV spherical irradiance levels in the absence (●) and presence (○) of visible light. Error bars represent 1 SE. All results were based on a UV exposure of 9 min at each spherical irradiance. Average normalized UV inactivation rate (Z value response in the absence of PR light) was $8.5 \times 10^{-4} \text{ cm}^2/\mu\text{W} \cdot \text{s}$.

Z value response, *B. subtilis* spores. To determine the effect of RH on airborne organisms in which DNA conformation does not change with hydration state, a Z value response was measured in aerosolized *B. subtilis* spores at both 50 and 95% RH. Results are shown in Fig. 4. In contrast to the vegetative cells tested, no difference in Z value response (independent *t* test, $\alpha = 0.05$) was observed when spores were suspended and UV irradiated in atmospheres maintained at these two RH levels. The Z value response for *B. subtilis* spores was less than that of vegetative cells at all RH levels.

Control for equivalent bioaerosol loading. Total microscopic counts were used as a control to ensure that approximately the same airborne bacterial concentrations were aerosolized and maintained throughout all experiments for a valid comparative basis. A comparison of total aerosolized bacteria concentrations, using an independent *t* test ($\alpha = 0.05$), confirmed that there were no significant differences between total bacterial cell removal rates for each experiment presented herein, regardless of the presence or absence of UV radiation or different RH levels. This observation confirmed that the deposition decay, as well as other removal mechanisms not directly measured, was the same for all experiments.

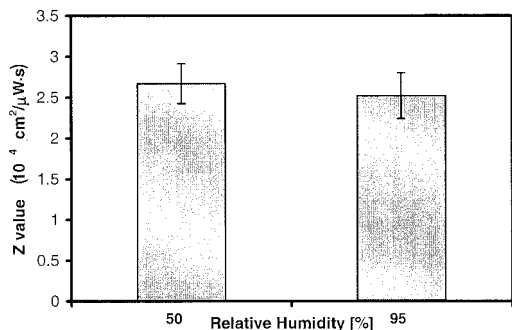


FIG. 4. Z value response determined for airborne *B. subtilis* spores at 50 and 95% RH. Average UV spherical irradiance was 7.5 ± 0.13 (SE) $\mu\text{W}/\text{cm}^2$. Error bars represent 1 SE.

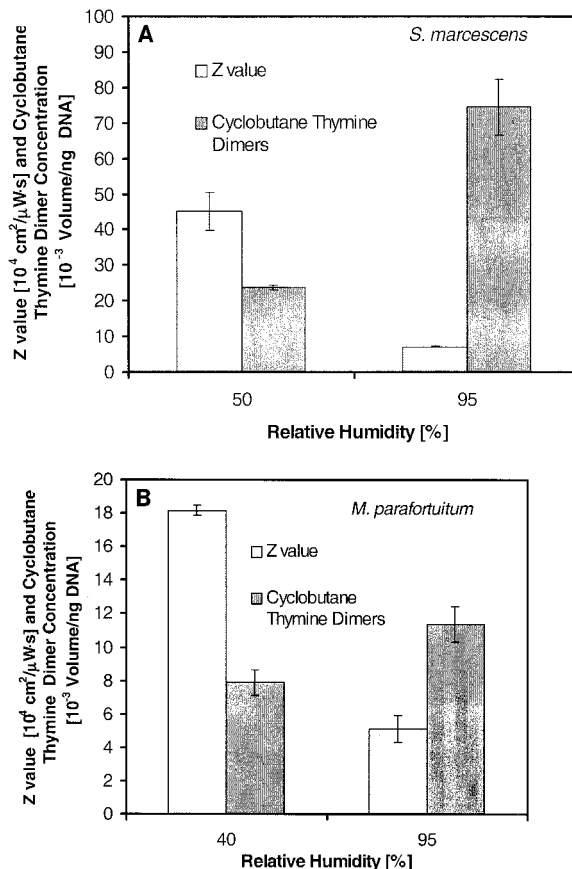


FIG. 5. Z value response and amounts of CTDs incorporated into DNA for UV-irradiated airborne *S. marcescens* (A) and *M. parafortuitum* (B). Average UV spherical irradiance was $7.5 \mu\text{W}/\text{cm}^2 \pm 0.13 \mu\text{W}/\text{cm}^2$. Error bars represent 1 SE. Volume represents the integrated intensity of all pixels in a defined area by immunofluorescence.

CTDs in airborne cells. CTD content was measured in airborne cells suspended in atmospheres containing RH levels that corresponded to maximum and minimum Z value responses observed in gram-negative *S. marcescens* (37) and acid-fast *M. parafortuitum* cells. Airborne *M. parafortuitum* demonstrated a twofold increase in CTD content in response to an RH increase from 40 to 95%. In similar experiments performed for *S. marcescens*, a threefold increase in CTD content was observed in response to an RH increase from 50 to 95%. For each bacterium, Z value response decreased as RH increased. All differences in Z value response and CTD content at 50 and 95% RH, as judged by independent *t* test ($\alpha = 0.05$), were significant. Results are presented in Fig. 5.

DISCUSSION

Effects of RH and UV dose in airborne bacteria. PR was observed and its rates were measured in *M. parafortuitum* cells suspended in both liquid and air. The PR rate associated with airborne *M. parafortuitum* cells increased with increasing RH and decreased with increasing UV dose.

As judged by Z value response during experiments performed in the presence and absence of photoreactivating light, the largest observed PR rate occurred when *M. parafortuitum*

cells were suspended in an atmosphere maintained at 95% RH. Below this maximum, PR rates decreased until they were below detectable levels, which occurred below 65% RH.

PR response was also determined to be sensitive to UV dose: there is a UV dose threshold beyond which PR will not occur regardless of RH levels. As a percentage of Z value response with no visible lights [$\% \text{ PR} = (Z_{\text{no light}} - Z_{\text{light}}) / Z_{\text{no light}}$], the highest PR rates were observed when the reactor averaged UV spherical irradiance levels were 1.88 and 3.7 $\mu\text{W}/\text{cm}^2$ (one and two lamps, respectively), and PR rate decreased as UV dose increased, until no PR was observed when the level reached 7.53 $\mu\text{W}/\text{cm}^2$ (four lamps operating). This irradiance level is slightly higher (ca. 35 $\mu\text{W}/\text{cm}^2$ for the upper level of a full-scale room) than the 25- $\mu\text{W}/\text{cm}^2$ irradiance previously recommended for full-scale UV applications for high-risk indoor environments (20).

Comparisons with previous literature. Despite the published reports of PR in organisms suspended in liquid (8, 21, 22, 52), insufficient data are available to draw quantitative comparisons between the airborne PR rates observed herein and those observed from liquid cultures. Some qualitative comparisons, however, are possible. In addition to *M. parafortuitum*, several other *Mycobacterium* species have a confirmed ability to photoreactivate; these species include *M. smegmatis* (11), *M. tuberculosis* H37Ra, and *M. marinum* (7). Gillis (11) previously studied PR in two species of *Mycobacterium* after their collection from UV-irradiated air. *M. phlei* showed no PR ability, while *M. smegmatis* demonstrated limited PR ability on agar plates following UV radiation in air at low (20 to 30%) and medium (50 to 60%) RH levels. Gillis (11) reports the only known attempt to observe PR behavior associated with airborne bacteria. The experimental design of this study, however, limited conclusive documentation that PR occurred in airborne bacteria because UV-irradiated cells were exposed to visible light when cultured on agar plates, not while suspended in air. While Gillis' study was limited by its light exposure scenario and culture conditions, it yielded some useful observations. For the same UV dose, limited PR was observed (approximately 15% culturability increase) at low and medium RH levels. While no PR was observed after UV irradiance at high RH, only a 10% culturability reduction was observed before visible light exposure, indicating that any PR would be difficult to measure given this overall low UV inactivation. When bacteria are allowed to photoreactivate on agar plates, the extent of PR may not apply to many common airborne scenarios. Following collection from UV-irradiated air, the immediate culturing of previously airborne bacteria on agar surfaces may induce DNA replication before cellular repair processes (including PR) are completed. The replication of potentially repairable DNA may result in the underestimation of the extent of PR. Beyond this study (11), no other airborne PR experiments have been reported in the literature.

Proposed mechanism for higher Z value responses at low RH. The following observations strongly suggest a fundamental biochemical mechanism that describes how RH influences the UV inactivation and photoreactivation of airborne cells: (i) the observed changes in Z value response in vegetative cells and lack of such response in spores, (ii) RH-dependent PR response, and (iii) CTD production in response to RH changes. The hydration of intracellular DNA appears to be

important in understanding UV inactivation-PR phenomena. In response to different RH levels, DNA experiences various degrees of hydration, which in turn dictate the physical conformation of this genetic material stored within a cell. Different physical DNA conformations have different photochemical responses and likely influence the overall response of prokaryotic cells to UV irradiation at different RH levels. At lower RH levels, thin prokaryotic DNA films experience a reversible transition to an A-like form (9). Transition to a more stable B form begins in the RH range between 50 and 65% and is completed above 75% RH (9). Conformational changes in DNA appear to dictate the type of UV-induced DNA damage it retains and are therefore most likely responsible for the increased inactivation rates observed at lower RH levels.

Previous UV research performed on thin prokaryotic DNA films indicates that the UV-induced DNA damage occurs in two dominant photoproduct types, depending on the RH (which in turn dictates DNA conformation). Under UV irradiation at 253.7 nm, a sharp increase in CTD content was observed above 65% RH and was the most dominant type of thymine lesion; presumably, the thymine dimer photoproduct is favored by the DNA B form (34, 36, 40, 53, 54). At low RH levels, irradiated DNA produced 5-thyminyl-5,6-dihydrothymine, often termed "spore photoproduct" (36, 40, 53, 54), and was the most dominant type of thymine lesion. The spore photoproduct has been reported only for UV-irradiated spores (31, 38, 53), isolated vegetative cell DNA at low RH (36, 41), isolated DNA bound to small acid-soluble proteins (SASPs) (as in spore DNA) (34, 51), and bacterial cells irradiated at -100°C temperature (54). Spore core water is between 10 and 30% of vegetative cell content (26), and spore DNA exists bound to SASPs that impart and maintain an A-like conformation to the DNA regardless of environmental conditions (51). Temperature, dehydration, and/or SASPs lead to a more A-like DNA conformation; when irradiated, this DNA form produces spore photoproducts in addition to other types of UV-induced DNA damage.

The relationships between Z value response and RH observed in whole airborne cells were consistent with those relationships between photoproduct distributions, DNA conformation, and RH reported previously in naked DNA films (40). The concomitant decrease in Z value response of airborne *M. parafortuitum* and *S. marcescens* with increases in CTD content through the RH range between 40 and 50% and 95% suggest that the UV inactivation-RH dependency is, in part, influenced by DNA conformation and its associated distribution of photochemical damage. Spore photoproducts increased to a maximum near 50% RH and then declined in an exponential fashion to an asymptotic minimum between 90 and 100% RH. Presumably, the spore photoproduct minimum and maximum CTD concentration correspond to the susceptibility of the microorganism suspended in liquid. CTDs are considered the most dominant type of DNA damage in liquid suspensions.

Little is known about the lethality of the spore photoproduct in vegetative cells. It appears that spore photoproducts are more lethal to vegetative cells than CTDs. At least three lines of evidence suggest a high inactivation potential associated with the incorporation of spore photoproducts in a vegetative cell genome. The first is associated with repair mechanisms. Although wild-type spores are approximately 10- to 100-fold

more resistant to UV than their vegetative counterparts (30, 34), mutations in spore photoproduct-specific repair pathways have yielded extremely UV-sensitive spores. Of the two spore photoproduct repair mechanisms known to exist in spores, only one, excision repair, is present in vegetative cells. This mechanism has also been demonstrated to repair CTDs (53). However, the only other known spore photoproduct repair mechanism, facilitated by the enzyme SP lyase, is unique to spores and operates only during spore germination. *B. subtilis* mutants engineered to remove these mechanisms have been found to be 40 times more susceptible to UV irradiation than the wild type (31). The DNA repair system in spores is more comprehensive and less error prone than the corresponding system in vegetative cells and confers much of the spores' heightened resistance to UV. Second, *Escherichia coli* cells have been shown to be less resistant to UV inactivation when irradiated frozen than when irradiated at room temperature (54). In these studies, the total amount of thymine altered was determined to be the same at each temperature. However, there was a decrease in CTDs and a concomitant increase in spore photoproducts in the frozen culture. This trend of decreasing inactivation potential with increasing CTD content (and RH) was also observed in this study. Finally, it appears that one major reason for the increased lethality associated with spore photoproducts is that they are produced in greater amounts than CTDs in bacterial DNA irradiated at 260 nm. These phenomena stem from the wavelength dependency for the stability of CTDs; they are formed most efficiently when DNA is irradiated at 260 nm. At a wavelength of 280 nm, the dimer form (CTDs) is favored (more stable); however, under irradiation at 240 nm, CTDs degrade into their monomer units of thymine bases. The stability and production of CTDs emanate from the differences in absorption spectra of the monomer and the dimer, as well as from the quantum yields for the formation and splitting of CTDs (13). The "photostability" of CTDs at 260 nm is manifested in only 15% of total DNA thymine being converted to CTDs in *E. coli* under very high UV doses, in contrast to the theoretical photochemical yield of 30 to 40%. Because spore photoproducts are not photoreversible at 240 nm, the theoretical yield (30%) can be achieved, and consequently twice as much thymine in UV-irradiated DNA undergoes damage when spore photoproducts are produced (13).

In accordance with the above observations, it was not surprising that RH changes had little effect on the UV inactivation potential of airborne *B. subtilis* spores. The proteins (SASPs) bound to the DNA did not allow for changes in DNA conformation in response to cell hydration (i.e., RH level), and thus they produced spore photoproducts as the dominant form of DNA damage in response to UV. Of important engineering consequence is the observation that the airborne PR rates of *M. parafortuitum* increased greatly when RH was above 80%. This increase was likely coincident with the complete transition of its DNA to a B form and presumably with the exclusive incorporation of CTDs into the genome as DNA damage. CTDs are a substrate for DNA photolyase, but spore photoproducts are not (33). Therefore, PR, as observed, is not a functional recovery mechanism at low RH levels. Although there was no analytical way to confirm the incorporation of spore photoproducts within airborne *M. parafortuitum* and *S. marcescens*, a marked increase in CTD content was confirmed,

as was CTD reduction in response to visible light exposure in liquid (data not shown).

Other water-mediated mechanisms may be important to understanding UV inactivation-RH relationships. Bacterial cells in a dehydrated state might also be expected to respond differently to UV inactivation due to the hydration and rehydration of proteins whose structures and catalytic functions intrinsically depend on their conformation and hence affect DNA dark repair enzymes and/or other metabolic functions.

Conclusions. PR of airborne bacteria was induced by artificial sunlight and substantially decreased the Z value response of *M. parafortuitum* (twofold decrease above 80% RH). PR in these experiments occurred at high RH levels and decreased with increasing UV dose. A threshold UV dose exists beyond which PR will not occur in airborne bacteria regardless of RH. The RH-dependent UV inactivation and PR rates observed were in agreement with the hypothesis that intracellular CTD content governs the UV inactivation response of airborne cells only at high RH levels (RH > 75%). Hydration of DNA and its subsequent conformational changes dictate the type of DNA damage incorporated into airborne cells irradiated with UV. A change in the type of UV-induced DNA lesions, from the CTD to another type of photoproduct (presumably the spore photoproducts), may be responsible for the greater UV susceptibility of airborne vegetative bacterial cells under dehydrated conditions.

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