Rapid Immunoassays for Detection of UV-Induced Cyclobutane Pyrimidine Dimers in Whole Bacterial Cells

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Immunoassays were developed to measure DNA damage retained by UV-irradiated whole bacterial cells. Active *Mycobacterium parafortuitum* and *Serratia marcescens* cells were fixed and incubated with cyclobutane pyrimidine dimer-binding antibodies after being exposed to known UV doses (254 nm). When both fluorescent (Alexa Fluor 488) and radiolabeled (^{125}I) secondary antibodies were used as reporters, indirect whole-cell assays were sensitive enough to measure intracellular UV photoproducts in *M. parafortuitum* and *S. marcescens* cells as well as photoenzymatic repair responses in *S. marcescens* cells. For the same UV dose, fluorescent DNA photoproduct detection limits in whole-cell assays (immunofluorescent microscopy) were similar to those in fluorescent assays performed on membrane-bound DNA extracts (immunoslot blot). With either fluorescent or radiolabeled reporters, the intracellular cyclobutane pyrimidine dimer content of UV-irradiated whole bacterial cells could be reliably quantified after undergoing a <0.5-order-of-magnitude decrease in culturability. Immunofluorescent microscopy results showed that photoenzymatic repair competence is not uniformly distributed among exponential-growth UV-irradiated pure cultures.

The response of prokaryotes to UV radiation is important for understanding how UV influences population dynamics in natural systems (11, 21) and the performance of engineered disinfection systems (10, 27, 30). UV performance in engineered systems has commonly been assessed on the basis of culturability changes using conventional enrichment techniques (7, 16, 19). While these assays provide a surrogate measure of the ability of UV radiation to render culturable bacteria nonculturable, only a small fraction of microorganisms in natural systems or engineered treatment systems are culturable (1). Additionally, conventional culturing assays cannot provide specific information regarding the types of DNA damage individual cells retain, the rate(s) at which DNA damage has been incurred, and the subsequent intracellular genetic repair (if any) that occurs in aquatic or aerosol environments.

The literature cites the cyclobutane pyrimidine dimer (CPD), the 6-4 photoproducts, and the 5-thymine-5-dihydrothymine (spore photoproducts) as the most common type of UV-induced DNA damage observed in UV-irradiated prokaryotic cells (18, 32, 39). In bacteria, the detection and measurement of UV-induced DNA photoproducts has predominantly relied either on chromatographic separation techniques using radiolabeled DNA bases or on indirect assays. Both techniques require the extraction and purification of DNA. To measure DNA lesions by chromatography, ^{14}C or ^3H must be added to microbiological enrichments to produce organisms with radiolabeled thymine bases incorporated into their DNA. Following UV exposure, DNA is extracted from irradiated cells and hydrolyzed to cleave its phosphodiester bonds, which produces individual nucleotides and UV-associated dimers; UV photoproducts are typically quantified by high-perfor-

mance liquid chromatography with a UV detector (17). Another chromatography-based method, ^{32}P postlabeling (high-performance liquid chromatography detection) (6), allows DNA damage determination without prior incorporation of radiolabeled bases into the microorganism DNA.

The development of monoclonal antibodies that are highly specific for the CPD has greatly increased the ease, sensitivity, and application of UV photoproduct detection. Several types of immune-based detection methods have been successfully applied to both eukaryotes and prokaryotes. Enzyme-linked immunosorbent assays have seen only limited application in CPD analysis because of the low reproducibility associated with immobilizing negatively charged DNA in plastic microtiter wells (14). A highly sensitive competitive radioimmunoassay (RIA) has been developed (18) to detect very low CPD quantities and has been useful in the study of sunlight-associated UV damage and repair in bacterioplankton and marine viruses (11, 20, 43). Quantification of UV photoproducts in membrane-immobilized DNA, where bound CPD antibodies are quantified by radiochemical, fluorescent, or enzyme-conjugated secondary antibodies, has been reported as a reliable technique. DNA damage has been detected and quantified in these immunoslot blot (ISB) systems utilizing very sensitive chemiluminescent detection (14), secondary antibodies conjugated to alkaline phosphatase enzymes (42), and secondary antibodies conjugated to radioactive iodine (^{125}I) (29). While those studies focused on measuring UV damage incorporated into prokaryotic genomes, Plaza and coworkers (29) demonstrated that CPD-binding antibodies could recognize DNA lesions incorporated into the genomes of whole mammalian cells by an indirect radiolabeled method.

In addition to advancing the fundamental understanding of nucleic acid photochemical behaviors (22, 24, 39, 40), modern DNA photoproduct detection techniques have been useful in elucidating the effects of environmental conditions on the types of stable photoproducts that prokaryotic cells retain

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when exposed to natural or artificial UV sources (27). Extraction and analysis of genomic DNA from a wide variety of environments have demonstrated the ability to track DNA photoproducts through a variety of natural and engineered conditions, which in turn has prompted a broad range of ecological implications and disinfection treatment criterion (15, 26, 33, 41, 43).

The need for genomic incorporation of radioactive DNA bases and associated culturability requirements, however, restrict the economy and robust use of chromatographic methods for UV radiation studies of bacteria in their environment. Further, the extraction and isolation of DNA for most immune-based DNA photoproduct analysis are time consuming, generally require relatively large amounts of starting material, and do not allow in situ observation of intracellular DNA damage. In response to these limitations, we report here the development and application of an immune-based technique that eliminates the need for extraction and uses CPD-specific antibodies to quantify DNA photoproducts in whole prokaryotic cells. Whole-cell antibody assays can allow the detection and quantification of UV damaged cells in situ, thus providing for spatial resolution of UV-damaged populations in environmental samples. This whole-cell immunoassay should increase the simplicity of DNA photoproduct analysis over its extraction-based counterparts and provides the potential for concurrent assessment of genomic DNA damage with whole-cell phylogenetic analytical techniques such as fluorescent in situ hybridization.

MATERIALS AND METHODS

Bacterial cultures and enumeration. Acid-fast *Mycobacterium parafortuitum* (ATCC 19689) and gram-negative *Serratia marcescens* (ATCC 13880) were used in the UV inactivation and photoenzymatic repair (PER) experiments. Cells for UV inactivation experiments were grown on agar plates containing soybean-casein digest agar (SCDA) (Difco Laboratories, Detroit, Mich.) and transferred to liquid suspensions as described previously (28). In conjunction with all immune-based assays for UV inactivation and PER experiments, culturable bacteria were quantified from batch liquid UV reactors. A modification of the standard spread plate count method was used to enumerate culturable bacteria: within 0.5 h after collection, samples were diluted (usually 1:10) in 50 mM phosphate-buffered saline (PBS; 150 mM NaCl, pH 7.2) solution and plated by a semiautomatic spiral dispensing method (Spiral Biotech, Inc., Bethesda, Md.) according to the manufacturer's recommendations. All plating used SCDA, and incubations were carried out at 37°C. Plates for *S. marcescens* were incubated for 24 h. At least three replicates of each sample were obtained and enumerated for all experiments. All plating was performed in indirect, dimmed light, and incubations were completely protected from light to control for PER. All plate count samples were stored for less than 0.5 h in distilled water in the dark at 4°C.

Whole-cell immunoassay preparation and primary incubation. Gram-negative *S. marcescens* cells were fixed by incubation in a 3:1 methanol-acetic acid mixture at -20°C for 2 min. Cells were centrifuged at $14,000 \times g$ for 5 min, and the supernatant liquid was then removed with a pipette. The pellet was washed in 70% ethanol at 4°C and stored for no more than 1 h. To complete the fixation step, cells were washed in a mixture of 0.1 M NaOH in 70% ethanol at room temperature and rinsed twice with PBST (10 mM phosphate buffer, 150 mM NaCl, 0.05% Tween 20 [pH 7.5]) by repeated centrifugation, decanting, and resuspension. Gram-positive *M. parafortuitum* cells were fixed by incubation with 10 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.) per ml in a 10 mM Tris-1 mM EDTA (pH 7.5) buffer for 0.5 h at 37°C. Cells were rinsed twice in PBST by repeated centrifugation, decanting, and resuspension.

Fixed cells were incubated with primary antibody (anti-cyclobutane thymine dimer mouse-derived monoclonal antibodies; Kamiya Biomedical Company, Seattle, Wash.) (20) in PBST containing 1% bovine serum albumin (Sigma Chemical Co.) at 22°C for 18 h on an orbital shaker at 150 rpm. Primary antibody incubation was performed at an optimized 1:2,000 (wt/vol) dilution, after which

FIG. 1. Petri dish reactor used to measure the effect of UV irradiance on the culturability and CPD content of whole bacterial cells suspended in liquid. Spherical quartz actinometry cells were mounted on the bottom of the dishes and immersed in the bacterial suspensions to measure the UV dose delivered.

cells were rinsed twice in PBST by repeated centrifugation, decanting, and resuspension.

Immunofluorescent microscopy (IFM). Intracellular CPD content was measured by immunofluorescent microscopy. Within whole cells, CPD-bound primary antibodies were subsequently exposed to fluorescently conjugated anti-mouse antibodies: A 1:10,000 (vol/vol in PBST) solution of goat-derived anti-mouse whole-molecule immunoglobulin G, conjugated to Alexa Fluor 488 dye (Molecular Probes, Eugene, Oreg.), was incubated with the whole cells following the primary antibody incubation. This secondary incubation continued for 3 h at room temperature in the dark on an orbital shaker at 150 rpm. The resulting intercellular fluorescing complex of primary and secondary antibodies, bound to UV-damaged DNA, was visualized and subsequently quantified by using modifications to widely accepted epifluorescent microscopy techniques (2, 3, 8, 9).

Cells stained with fluorescent antibodies were counterstained with the DNA intercalating agent 4',6'-diamidino-2-phenylindole (DAPI) (Sigma Chemicals). Cells were filtered onto black polycarbonate membranes (average pore size, 0.2 μ m; Poretics, Inc., Livermore, Calif.) and rinsed with 50 ml of sterile deionized water to remove any unbound secondary antibodies. Once filtered, they were stained for 1 min with DAPI at a final concentration of 1.0 μ g/ml and washed with 50 ml of filter-sterilized deionized water. Cells were then viewed with a Nikon epifluorescent microscope equipped with a HQ470/40 excitation filter, HQ525/50 emission filter, and Q495LP beam splitter (ChromaTechnology Corp., Brattleboro, Vt.) for Alexa Fluor 488 fluorescence and with a D360/40 excitation filter, 420 emission filter, and 400DCLP beam splitter (ChromaTechnology Corp.) for DAPI fluorescence. Antibody- and DAPI-conferred fluorescence were separately quantified with Simple PCI image analysis software (Compix Inc. Imaging Systems, Cranberry Township, Pa.) using a set gray-level threshold. A 32-bit cooled, color digital camera (Spot camera; Diagnostic Instruments, Sterling Heights, Mich.) captured fluorescent micrographs, which were archived and printed using Adobe Photoshop software (Adobe systems, San Jose, CA). The IFM signal was quantified by total pixel intensity per cell (normalized by DAPI direct counts). Averages of pixel intensity and cell counts were based on observations of five images and more than 1,000 total cell counts from each sample.

IRM. Secondary antibody incubations were also performed with a sheep derived anti-mouse immunoglobulin G whole molecule conjugated to 125 I (Amersham Life Science Ltd., Little Chalfont, England). Incubations were performed at an activity of 0.5 μ Ci/ml under incubation conditions previously described for fluorescent Alexa Fluor 488 antibody conjugates, excluding these fluorescent antibody conjugates. The same whole-cell protocol was used as described above, except that cells were repeatedly centrifuged and decanted to remove unbound radioactivity. The washed cells were resuspended in 1 ml of PBST, and 200 μ l was reserved for direct microscopic analysis while the remaining 800 μ l was added to scintillation vials containing 10 ml of scintillation cocktail (ScintiSafe Plus; Fisher, Fair Lawn, N.J.). 125 I disintegrations were counted with a model 2300TR scintillation analyzer (Packard Instruments, Downers Grove, Ill.). Total disintegrations (counts per minute) were normalized to total bacteria present, as determined by direct DAPI counts. Immunoradiometric assay (IRM) results were recorded in counts per minute per cell.

To compare the sensitivity of the IFM method with that of the ISB method, *S. marcescens* was UV irradiated at a dose of 23 J/m². DNA was extracted and CPD signal quantified with Alexa Fluor 488 as previously described (28).

UV inactivation and PR experiments. Liquid inactivation and PER experiments were performed in a class II biosafety cabinet to maintain constant environmental and aseptic conditions (Labconco Inc., Kansas City, Mo.). Sterile

FIG. 2. Epifluorescent micrographs of UV-irradiated *S. marcescens* cells displaying immunofluorescence associated with intracellular CPD content. IFM signal is conferred by antibody complex conjugated to Alexa Fluor 488. Images correspond to increases in UV exposure between 0 and 20 min; the associated decrease in culturability of *S. marcescens* is presented in Fig. 4. Magnification, $\times 1,000$.

deionized water (70 ml) containing approximately 10^6 CFU/ml of mid-exponential-phase *S. marcescens* cells was placed in a 100- by 15-mm sterile plastic uncovered petri dish (Fisher Scientific, Pittsburgh, Pa.). The test suspensions were continuously mixed on a magnetic stir plate at approximately 100 rpm by a 1-cm-long Teflon-coated stir bar immersed in the petri dish. Full-spectrum, visible-light lamps (F40T12SUN; Verilux, Inc., Stamford, Conn.) and UV lamps (G30T8; Osram-Sylvania, Hanover, Mass.) were positioned in the biosafety cabinet 0.6 m from the petri dish for liquid batch experiments. The UV lamps were wrapped with aluminum screen coverings (Research Products, Madison, Wis.) to control the UV flux delivered to the suspended bacteria (27). UV lamp spectral power distributions were determined between 240 and 400 nm using a scanning radiometer (model ISADHL; Jobin Yvon/Horiba Inc., Edison, N.J.) (28). The spectral power distribution measured for the UV lamps with screens showed that 95% of power was emitted at 253.7 nm after 100 h of operation. The UV dose delivered to the bacterial suspensions was measured directly by quartz actinom-

etry spheres, which were permanently mounted on the bottom of the petri dishes and submerged in the bacterial suspensions (Fig. 1); this provided an absolute UV radiation measurement integrated over the depth of the suspensions. UV radiance is reported as a spherical radiance in accordance with previously described actinometry methods most appropriate for estimating UV exposures to bacterial cells (26, 31, 34). The average UV spherical radiance was 0.075 ± 0.003 W/m² (mean $\pm$ standard error).

Liquid-based UV-inactivation and PER rates were determined as follows. Bacteria were suspended in plastic petri dishes at a known UV spherical radiance (0.075 W/m², as determined by actinometry), and initial concentrations of total and culturable bacteria were determined before the UV lamps were started. Bacteria were UV irradiated in the dark, and samples were taken until an ~ 2 -order-of-magnitude reduction in culturability was observed. The UV lamps were then turned off, and visible lights were turned on. Samples were taken after 10, 20, 30, 45, 60, and 90 min of visible-light exposure. PER control experiments

FIG. 3. Fluorescent ISB analysis of DNA extracted from UV-damaged *S. marcescens* cells from three separate UV irradiation experiments. The fluorescent reporter-antibody complex (Alexa Fluor 488) was quantified as an image volume (integrated intensity of all pixels in a defined area). Data are for *S. marcescens* irradiated at a dose of 23 J/m².

were executed in a similar manner, except that post-UV samples were taken in the dark. Samples represent a time series of total and culturable cell concentrations taken during all bench-scale liquid experiments. First-order UV inactivation rate coefficients were used to estimate the exponential decay of cells within the reactor (corrected for natural decay rate by no-UV controls) according to a previously described completely mixed batch reactor model (28).

To estimate variability in UV inactivation rate and CPD production rate, all experiments were executed in multiple independent trials. All average concentrations (CFU per milliliter) for each treatment scenario were logarithmically transformed (natural log base) and pooled, and the rate coefficients were estimated by the least-squares method for determining the best fit to the data. Rates of CPD production were determined in a similar manner, except that fluorescent intensity and scintillation data were not logarithmically transformed. 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 rates estimated for different experimental treatments, a method of dummy variables was used (3, 13).

FIG. 5. IRM measurements of intracellular CPD content in whole *S. marcescens* cells sequentially irradiated by UV and visible light. IRM increased with increasing UV exposure; relative CPD ($\diamond$) increases were concomitant with losses in culturable *S. marcescens* cells ($\blacklozenge$). After 10 min, UV exposure was stopped and *S. marcescens* cells were exposed to full-spectrum visible light. Immediate and rapid CPD decline and concomitant culturing increases observed under visible-light exposure suggest PER occurrence. ¹²⁵I decay was normalized to total cell concentration (DAPI counts) as determined by direct microscopic counts. The UV spherical irradiance was 0.075 ± 0.003 W/m².

RESULTS

The relative amount of intracellular DNA photoproducts was quantified by using IFM signal intensity and increased as UV dose increased. Figure 2 depicts CPD-associated fluorescence in UV-damaged *S. marcescens*. A similar IFM response was also observed for whole *M. parafortuitum* cells. The whole-cell assay, with Alexa Fluor 488 as a reporter, could reliably measure CPD in *S. marcescens* after a minimum UV dose of 23 J/m². Using a common low-light charge-coupled device camera for this application required the collection and assay of approximately 1.0×10^5 UV-irradiated cells (corresponding to 4 ng of photoproduct-containing DNA). For the same UV dose,

FIG. 4. Whole-cell IRM ($\bullet$) and IFM ($\circ$) measurements of intracellular CPD content in *S. marcescens* increased in response to increasing UV exposure; CPD increases were concomitant with culturability loss as determined by plate counts ($\blacktriangle$). The dashed line represents a linear regression for the plate counts. The UV spherical irradiance was 0.075 ± 0.003 W/m². Pixel intensity for fluorescent measurements and counts per minute for radioimmune response were normalized to total cell concentration as determined by direct microscopic counts.

FIG. 6. Measurement of relative intracellular CPD content and PER response by IFM (hatched bars) and plate counts (shaded bars). Pre-PER and post-PER, data obtained immediately after a UV dose of 4.5 J/m² and data from the same population after a 90-min exposure to artificial sunlight. Error bars represent 1 standard error (three experiments).

the ISB method, which requires membrane bound DNA extracts, demonstrated a slightly better sensitivity than the IFM: DNA photoproducts could be detected in as little as 2 ng of extracted DNA, which corresponded to approximately 5.0×10^4 cells. The range of linear responses for the fluorescent dye used was approximately 1 order of magnitude, and this range was demonstrated by using ISB analysis for membrane-bound DNA (Fig. 3).

The IFM and IRM methods were compared for the ability to quantify changes in intracellular CPD content during UV inactivation and subsequent PER. Figure 4 summarizes the correlations observed between increases in whole-cell immune-based DNA photoproduct signals (fluorescent and ¹²⁵I) and concomitant decreases in the culturability of *S. marcescens*. The specific rate of CPD incorporation into whole-cell DNA was estimated as first order: IFM analysis of DNA-damaged whole cells recorded a $0.083 \pm 0.020 \text{ h}^{-1}$ rate coefficient, and IRM scintillation counting of the cells from parallel experiments using ¹²⁵I labeled photoproducts demonstrated a photoproduct incorporation rate coefficient of $0.086 \pm 0.014 \text{ h}^{-1}$. These differences were not statistically significant at a 95% confidence level, as judged by comparing the first-order decay data (13). The inactivation efficiency of CPD incorporated by UV-irradiated *S. marcescens* cells was approximately a 2.1 time increase in signal (relative CPD content) for each order-of-magnitude decrease in culturability.

Both IFM and IRM assays were used to assess PER responses in whole *S. marcescens* cells. Following UV irradiation that rendered a significant amount of the cells introduced to the petri dishes nonculturable (a >2-order-of-magnitude reduction), cells were exposed to visible light for nearly 90 min, during which time relative intracellular CPD content was concurrently measured by IFM and IRM. Results summarized in Fig. 5 confirm the sensitivity of whole-cell IRM to measure visible-light-activated genomic repair. At the experimental point where UV exposure ceased and visible-light exposure commenced, the rapid decline in *S. marcescens* culturability ended, and a marked change to a (positive) recovery in plate

counts followed (fourfold increase). Concomitant with this recovery was a significant decrease in intercellular CPD content, which confirmed the ability of the IRM to measure PER response in whole bacterial cells (Fig. 5). A set of similar experiments also confirmed the ability of IFM to measure PER response in whole cells. The decrease in total pixel intensity corresponded with an increase in culturable bacteria (Fig. 6). IFM PER responses were digitally recorded and reproduced with photographic imaging software (Adobe Photoshop 5.0) in Fig. 7. Total cells and UV-damaged cells could be separated into colors corresponding to the different fluorochromes used. Green pixel intensity (a measurement of CPD concentration reported by Alexa Fluor 488) was normalized to total numbers of DAPI-stained cells. The digitized epifluorescent micrographs presented optically confirm the intracellular repair of CPD. Only a portion of UV-irradiated cells responded to visible light: approximately 50% of UV-irradiated cells were able to remove CPD, while the CPD concentration in other cells remained unchanged.

DISCUSSION

An immune-based method for the detection of relative CPD content inside whole prokaryotic cells was developed and tested. Using either fluorescent (IFM) or radiolabeled (IRM) antibodies as reporters, this method was capable of measuring, in situ, the production of intracellular CPD during UV inactivation and their subsequent removal during PER. While ISB assays and the competitive RIA reported thus far exhibit very low CPD detection limits (14, 29, 42), they require DNA extraction and purification, which can be problematic in some environmental samples. With *S. marcescens*, a similar range of CPD sensitivity was observed when IFM (detection limit, approximately 1.0×10^5 cells containing 4 ng of DNA), which requires no DNA extraction, was compared to an ISB method (detection limit, approximately 5.0×10^4 cells containing 2 ng of extractable DNA suitable for analysis). For functional interpretations, CPD quantities must be normalized to the amount of host DNA. In whole-cell applications (IFM and IRM), this is easily accomplished by normalizing signal to total bacterial counts using DNA-intercalating agents; in this study we used DAPI, but any DNA-binding stains would be suitable provided that their emission spectra can be separated from the color emitted by their secondary antibody conjugate. For the ISB method, DNA extraction and analytical preparation require protein separation and subsequent DNA precipitation: when low quantities of DNA are handled, its purification can have low efficiencies and may be complicated by interactions with metals or their complexes, which are often present in surface and groundwaters (e.g., iron). This can be a serious interference in environmental samples. Conventional DNA absorbance measurements typically have detection limits in the microgram-per-milliliter range, which can be improved to 10 ng/ml by binding with DAPI (12). These detection constraints set practical limits on the numbers of cells required for efficient DNA extraction and measurement to more than the 5×10^4 cells previously mentioned. When limited amounts of cells can be concentrated from environmental samples, DNA extraction efficiency is low, or there is high potential for interference with DNA purification, whole-cell immunoassays such as IFM and

FIG. 7. Epifluorescent micrographs of UV-irradiated *S. marcescens* cells from pre- and post-PER experiments. Green fluorescence was emitted by CPD-antibody complexes (IFM) conjugated to Alexa Fluor 488 and corresponds to the extent of intercellular DNA damage. Blue fluorescence corresponds to intracellular DNA-DAPI complexes, which corresponds to the DNA in all bacteria present. Magnification, $\times 1,000$.

IRM may offer attractive analytical alternatives for UV-induced DNA damage assays with a detection limit near that of ISB-type immunoassays. The IFM and IRM assays described here can detect the total amount of CPD in a suspension of cells or can be used to detect the fluorescence (as a surrogate measurement of CPD-associated antibodies) of a single cell or group of cells using epifluorescent microscopy and image analysis. For IFM and IRM, detection required approximately 10^5 and 10^7 cells, respectively, given UV doses that have been shown to inactivate a broad range of waterborne (4, 5, 7, 16, 35) and airborne (19, 23, 26, 36) bacteria under common environmental conditions.

The IFM technique was useful for rapid assessment and visual observations of CPD damage in UV-irradiated cells and can be adapted for analysis of environmental sources containing relatively low bacterial concentrations. However, the extent of linear responses observed from common fluorochrome dyes used in this application is relatively low (in this study, approximately 1 order of magnitude), and because of background separation issues and the variable quality and type of image analysis hardware and software, the reproducibility of fluorescence measurements from the analytical imaging of whole cells can be problematic. IRM provides a much larger linear response range; liquid scintillation analysis has a simple protocol,

and background separation is rarely problematic in microbiological applications.

Both IFM and IRM were useful in quantifying the removal (repair) of CPD during PER. The IFM assay was accurate for measuring intracellular DNA damage through the multiple logarithmic reductions that are often required for UV inactivation of microorganisms in water and wastewater treatment systems and in air systems; however, the more sensitive IRM was required to quantify the lower rates associated with CPD repair as in PER. For DNA damage measurements in relatively low UV fluxes associated with many natural systems, the ISB, using highly sensitive reporters (42), or the competitive RIA (43) yields clearer delineation between DNA damage incorporated at different sublethal doses.

The IFM method allows visualization of cells that contain a specific type of UV-induced DNA damage. This technique could be useful for studying the spatial distribution of UV damage in many environments and/or in linking genomic damage with ecological investigations (16S rRNA probe hybridizations [fluorescent in situ hybridization]) (25).

Figure 7 is a characteristic image representing the data used in the determination of the relative CPD content in Fig. 6. Approximately 50% of the UV-irradiated organisms were able to remove CPDs (based on a color change from green to blue). This represents a visual approximation and does not account for partial removal of CPD content (i.e., cells may have removed a fraction of CPDs). The more quantitative approach accounts for partial CPD removal. With image analysis and IFM, this approach revealed that 62% of CPDs were removed (Fig. 6), and with IRM, a 50% removal of relative CPD content (Fig. 5) was observed. These results are similar (standard errors of IRM and IFM assays range from 10 to 15%).

Liquid-based PER results provided some insight into the PER-UV dose relationship observed here, as well as into previous observations of "dose reduction" (16, 37, 38). In addition to confirming that CPDs are removed during PER (Fig. 5 and 6), IFM indicated that only a fraction of UV-damaged cells have any DNA repair response to visible light (i.e., concentrations of intracellular CPD decrease), although all the cells ostensibly had competent photolyase enzyme systems capable of CPD repair. As UV dose increased, the fraction of cells that were unable to perform PER increased; thus, an overall decrease in PER-competent culturable cells resulted (i.e., dose reduction). This observation is in contrast to a scenario where the PER responses occurred in most cells of a monoculture subjected to UV inactivation.

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