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Diverse Responses to UV-B Radiation and Repair Mechanisms of Bacteria Isolated from High-Altitude Aquatic Environments▽

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***Acinetobacter johnsonii* A2 isolated from the natural community of Laguna Azul (Andean Mountains at 4,560 m above sea level), *Serratia marcescens* MF42, *Pseudomonas* sp. strain MF8 isolated from the planktonic community, and *Cytophaga* sp. strain MF7 isolated from the benthic community from Laguna Pozuelos (Andean Puna at 3,600 m above sea level) were subjected to UV-B (3,931 J m^{-2}) irradiation. In addition, a marine *Pseudomonas putida* strain, 2IDINH, and a second *Acinetobacter johnsonii* strain, ATCC 17909, were used as external controls. Resistance to UV-B and kinetic rates of light-dependent (UV-A [315 to 400 nm] and cool white light [400 to 700 nm]) and -independent reactivation following exposure were determined by measuring the survival (expressed as CFU) and accumulation of cyclobutane pyrimidine dimers (CPD). Significant differences in survival after UV-B irradiation were observed: *Acinetobacter johnsonii* A2, 48%; *Acinetobacter johnsonii* ATCC 17909, 20%; *Pseudomonas* sp. strain MF8, 40%; marine *Pseudomonas putida* strain 2IDINH, 12%; *Cytophaga* sp. strain MF7, 20%; and *Serratia marcescens*, 21%. Most bacteria exhibited little DNA damage (between 40 and 80 CPD/Mb), except for the benthic isolate *Cytophaga* sp. strain MF7 (400 CPD/Mb) and *Acinetobacter johnsonii* ATCC 17909 (160 CPD/Mb). The recovery strategies through dark and light repair were different in all strains. The most efficient in recovering were both *Acinetobacter johnsonii* A2 and *Cytophaga* sp. strain MF7; *Serratia marcescens* MF42 showed intermediate recovery, and in both *Pseudomonas* strains, recovery was essentially zero. The UV-B responses and recovery abilities of the different bacteria were consistent with the irradiation levels in their native environment.**

DNA damage induced by UV radiation (UVR) is wavelength dependent: UV-A (320 to 400 nm) causes only indirect damage to DNA, proteins, and lipids through reactive oxygen intermediates. On the other hand, UV-B (280 to 320 nm) and UV-C (100 to 280 nm) cause both indirect and direct damage because of the strong absorption at wavelengths below 320 nm by the DNA molecule. The most abundant products formed by irradiation with UV-B are cyclobutane pyrimidine dimers (CPD) (22). Bacteria have several repair mechanisms in response to UVR-induced damage. These mechanisms are usually classified into dark repair (DR) and photoreactivation. There are three different dark repair mechanisms described here: (i) nucleotide excision repair, (ii) postreplication recombinational repair, and (iii) error-prone repair. All mechanisms are inducible as part of the SOS regulon, and the induction is dependent on DNA damage (24–26, 29). On the other hand, photoreactivation is a light-dependent repair mechanism that uses a photolyase enzyme that can be activated by different wave lengths, such as UV-A (315 to 340 nm) and photosynthetic active radiation (PAR) (400 to 700 nm). Therefore, both wavelengths should be tested in studies of photorepair in order to determine the best one for cell reactivation. As a consequence, solar UV-B has the potential to cause negative effects on aquatic organisms, especially on bacterioplankton, since bacteria have simple haploid genomes with little or no functional redundancy and they are small, which precludes effective cellular shading and reduces the benefits of protective pigmentation (9). Considering that bacteria may account for up to 90% of the cellular DNA in aquatic environments, that these organisms play a central role in the cycling of nutrients, and that they constitute a fundamental link in carbon flow (i.e., the microbial loop [4]), the study of the influence of UV radiation on bacteria in the environment is of primary importance (14, 15). Several studies of UV irradiation and DNA repair in bacteria present in diverse aquatic environments have been carried out (1–3, 9, 12–14, 19, 27, 28, 30). However, most of these studies have been carried out at sea level. UV radiation increases with altitude. In one study, it was determined that UV radiation increased by 19% for every 1,000 m of altitude (5). Thus, bacteria living in high-altitude environments can be expected to show a wide range of resistance mechanisms. Some studies have been carried out in the Alps at altitudes between 1,000 and 3,000 m above sea level (asl). Warnecke et al. (30) have determined the abundance, identity, and growth state of *Actinobacteria* in Alpine mountain lakes with different UV transparencies. Sommaruga et al. (27) determined the effect of solar UV-B impact on the microbial food web in an Alpine lake. In that way, the Altiplano region in the Andes (also known as Puna) offers several advantages for this type of study. Water bodies can be found at higher elevations than in the Alps (up to 4,500 m) and at lower latitudes (22 to 28°S). Both variables, high elevation and lower latitude, would cause higher irradiation exposure and, therefore, a wider range of adaptive strategies in bacteria. The extreme conditions in these environments may be relevant for exobiology.

In a previous study, we isolated UV-B-resistant bacteria

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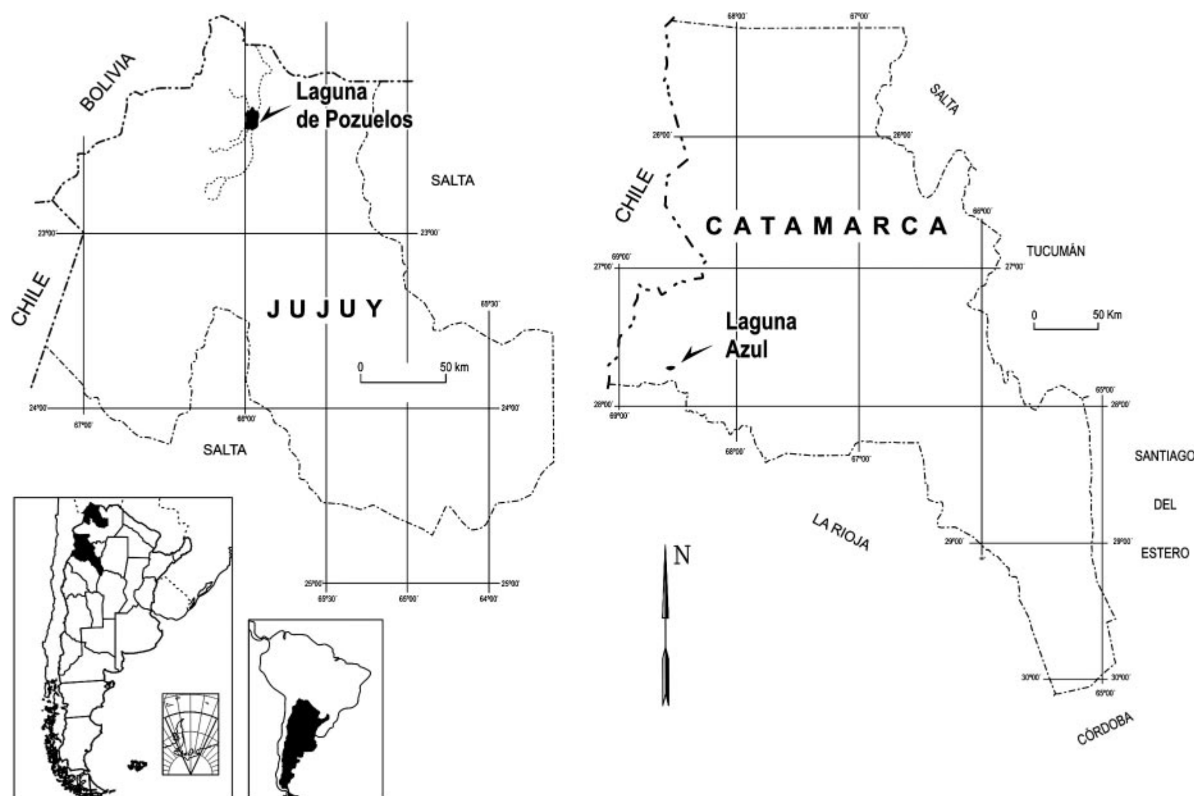

FIG. 1. Geographical locations of the high-altitude Andean wetlands used as sources of bacterial cultures: Laguna Pozuelos in Jujuy (Andean Puna) and Laguna Azul in Catamarca (Andean mountains).

from Laguna Azul, a pristine and remote wetland at 4,560 m asl, and we found a predominance of gram-positive bacteria. Both *Nocardia* sp. strain A5 and *Acinetobacter johnsonii* A2 (the single γ-proteobacteria isolated in that study) showed very high levels of resistance (8).

In the present work, we go further by determining the UV-B resistance and repair ability of bacteria isolated from Laguna Azul (including *Acinetobacter johnsonii*) and Laguna Pozuelos (found at lower altitude but also at lower latitude). These wetlands are of special interest because (i) they are pristine, (ii) they are located at high altitude (ranging from 3,600 to 4,560 m), (iii) they are surrounded by deserts, implying that no clouds will screen the increased UV irradiation, (iv) they are oligotrophic, resulting in relatively deeper penetration of UV in the water, and (v) they are shallow (20 to 120 cm deep). The isolates, therefore, should show a range of resistance and repair mechanisms compatible with the irradiation levels in their original environments. To test this hypothesis, we compared their UV resistance and repair ability with those of a marine *Pseudomonas putida* strain, 2IDINH, and *Acinetobacter johnsonii* ATCC 17909, a culture collection strain.

MATERIALS AND METHODS

Study area. The northwest of Argentina is particularly rich in wetlands located in the Puna and Andean regions at altitudes in the range of 3,000 to 4,600 m asl. Most of these wetlands are completely isolated, experience a wide daily range in temperatures, are slight saline to hypersaline, and are subject to a high intensity of solar UV-B radiation. The bacteria studied in the present work were isolated from two high-altitude wetlands.

Laguna Pozuelos is located at 22°19′S and 66°00′W in northwestern Jujuy province in the Argentinean Andes (Fig. 1). It is the main water body in an extensive internal drainage basin located at 3,600 m asl. Its total surface area is around 70 km^2, and its maximal depth is 80 cm in its central area. Vegetation is scarce. The basin is included within the network of Biosphere Reserves of UNESCO since 1990. The climate of the basin is cold and semiarid, with an average annual temperature below 9°C. The weather is markedly seasonal, with a rainy season in the summer (between December and March) and a dry season throughout the rest of the year. In accordance with the dry and rainy seasons, the surface of the lake is subject to an annual contraction-expansion cycle. In the sampling day in the austral autumn, the maximal UV-B irradiance reached 5.5 W m^{-2} for the 300- to 325-nm range.

Laguna Azul is an oligotrophic lake located at 4,560 m asl. It is part of the Salar de la Laguna Verde in the Andean region of Catamarca province, Argentina (27°34′S, 68°32′W). The location is a very isolated site with no access roads (Fig. 1). Rainfall is scarce, and the lakes are shallow and present a high metal content (19). At noon on the sampling day, in the austral summer, maximal UV-B irradiance reached 10.8 W m^{-2} for the 300- to 325-nm range.

A large collection of bacterial strains was isolated from the environments described above. Two of the γ-proteobacteria were selected for the present study. *Pseudomonas* sp. strain MF8 and *Serratia marcescens* MF42 were chosen as representative planktonic members of Laguna Pozuelos (3,600 m asl), since γ-proteobacteria constitute an important portion of the Pozuelos community (28% of clones in a clone library and 20% of fluorescent in situ hybridization counts) (M. E. Farías et al., unpublished data). *Acinetobacter johnsonii* A2 was chosen as the only γ-proteobacterium isolated from Laguna Azul because it was shown to be very resistant to UV-B in a previous study (8). *Cytophaga* sp. strain MF7 was selected as a benthic isolate that could be expected to be less exposed to UV radiation than the Pozuelos planktonic isolates and, therefore, should have less resistance and fewer repair capabilities (Table 1). Finally, we selected two strains from the culture collection for comparison: marine *Pseudomonas putida* 2IDINH and *Acinetobacter jonhsonii* ATCC 17909. The *Pseudomonas* genus has resistance and repair mechanisms that have been well studied (16–18, 23, 25). In addition, the marine *Pseudomonas putida* strain 2IDINH was chosen

TABLE 1. Phylogenetic affiliation, isolation origin, NCBI accession number, survival percentage, and CPD/Mb content after 3,931 J m^{-2} UV-B[a]

Strain	Origin	Closest relative	Accession no.	D_{37} (Jm^{-2})	r^2	No. of CPD/Mb	Photo repair with PAR		Photo repair with PAR plus UV-A		Dark repair	
							CFU	CPD	CFU	CPD	CFU	CPD
A2	Azul (plankton)	*Acinetobacter johnsonii*	AY963294	4.25 (0.37)	0.97	80.0	+++	++	+++	++	+++	−
17909	ATCC	*Acinetobacter johnsonii*	AB008692	3.30 (0.83)	0.83	160.0	+++	+++	+++	+++	−	−
MF8	Pozuelos (plankton)	*Pseudomonas* sp.	AF509476	4.11 (0.51)	0.96	44.3	−	−	+	+	−	−
2IDINH	Mediterranean Sea	*Pseudomonas putida*	AF307868	2.71 (0.39)	0.92	23.4	−	−	−	−	−	−
MF7	Pozuelos (benthos)	*Cytophaga* sp.	AF509475	3.25 (1.06)	0.98	400.0	+	++	−	++	+++	+++
MF42	Pozuelos (plankton)	*Serratia marcescens*	AM259579	3.95 (0.04)	0.89	21.6	+	+	+	+++	−	−

[a] Light (P and PA) and dark recovery of UV-B-irradiated cells, measured by CFU and CPD, is shown. + + +, recovery to values previous to UV-B exposure; + +, recovery to more than 50% of initial values; +, recovery to less than 50% of initial values; −, no recovery. Values are the averages from three independent experiments. D_{37} is the radiation required to inactivate 63% of a bacterial population. The D values were calculated from the regression line of the exponential decrease of CFU with time. The standard deviation of the linearized regression is shown in parentheses.

from the sea surface as a control that could be compared to the high-altitude *Pseudomonas* sp. strain MF8. *Acinetobacter johnsonii* ATCC 17909, in turn, was chosen for comparison with the congeneric Laguna Azul strain.

Isolates were maintained on Luria-Bertani agar medium (LB). Cultures were grown at 30°C in flasks containing 300 ml of LB with shaking (200 rpm). Fifty-milliliter portions of overnight cultures were used as inocula. Growth was determined by measuring the optical density at 600 nm. Cells were harvested in the mid-exponential phase by centrifugation for 30 min at 13,000 rpm at 4°C (RC5C; Sorvall Instruments).

UV-B irradiation. Since bacteria were isolated from oligotrophic environments, irradiations were performed under starvation conditions. Even though not exactly the same, the starvation conditions seemed more appropriate than active growth. Pellets were washed twice in 0.9% NaCl (wt/vol). The resulting preparation was incubated at 4°C overnight in order to reach a complete starvation state. Aliquots of each cell suspension were transferred to 45-ml sterile quartz tubes. The tubes were covered with an acetate sheet to block out UV-C and placed and incubated at 13°C with slow shaking (25 rpm). The tubes were exposed to a UV-B lamp (lamp 09815-06; Cole-Parmer Instruments Company) for 20 min. UV-B irradiance was quantified with a radiometer (radiometer 09811-56, Cole-Parmer Instrument Company), and the average intensity was 3.3 W m^{-2}. The UV-B dose after 20 min of exposure was 3,931 J m^{-2}. D_{37} values indicate the radiation required to inactivate 63% of a bacterial population (2). The D_{37} values were calculated from the regression line of the exponential decrease of CFU with time. The r^2 of the regression is provided as an indication of the goodness of fit.

Photoreactivation and dark repair. To determine the repair properties of the bacteria, UV-B-irradiated cell suspensions were subjected to three different treatments: (i) photoreactivation with PAR (P treatment), (ii) PAR plus UV-A (PA treatment), and (iii) darkness (DR treatment). The respective quartz tubes were covered with Mylar-D film, transmitting UV-A (315 to 400 nm) plus PAR (400 to 700 nm) (PA treatment) or Plexiglas filter UF-3, so that samples received only PAR (P treatment). The amount of UV-A radiation was measured using a Lutron radiometer (UV-340), and the intensity was 3.65 W m^{-2}. The intensity of PAR was 13.44 W m^{-2}, and it was measured using a Lutron radiometer (LX-170). The contribution of the dark repair system to recovery was studied by covering the quartz tubes with aluminum foil.

Cell suspensions were continuously shaken at 25 rpm and 13°C during the exposure. All treatments were done in duplicate. Three independent experiments were run for each bacterium, and mean values and standard deviations were determined.

Viable counts. A 0.1-ml sample of each cell suspension was removed after each treatment in order to prepare serial dilutions in 0.9% NaCl. Samples (50 μl) of the appropriate serial dilutions were spread on duplicate LB petri dishes. Rich medium was used to allow the repair mechanisms that require protein synthesis. The numbers of CFU were determined after 24 h of incubation in dark at 30°C.

A nonirradiated control run in the dark was carried out, and the numbers of CFU were also determined. All counts were done in duplicate.

DNA extraction and quantification. At the end of each incubation, samples were filtered through 0.22-μm filters (Isopore polycarbonate membrane filters, 47 mm) and stored at −70°C until CPD analysis.

For quantification of DNA damage, filters with bacteria were incubated for 30 min with 0.75 ml of 2% [wt/vol] CTAB isolation buffer (Sigma) (1.4 M NaCl, 0.2% [vol/vol] 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl, pH 8). After extraction, samples were washed with 0.5 ml chloroform-isoamyl alcohol (24:1, vol/vol) and centrifuged (10 min, 12,000 rpm). DNA was precipitated with 0.5 ml of isopropanol (1 h, 4°C) followed by centrifugation (30 min, 12,000 rpm, 4°C). The pellet was washed with 80% cold ethanol, vacuum dried, and dissolved in 0.1 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Samples were stored at −20°C. The DNA concentration was quantified fluorometrically using Hoechst 33258 dye.

Quantification of CPD. The amount of CPD was determined using the H3 antibody directed mainly against thymine dimers. Heat-denaturated DNA samples (100 ng) were blotted (Minifold I SRC96D dot blot apparatus; Schleicher & Schuell) onto nitrocellulose membranes (Protran, 0.1 μm; Schleicher & Schuell). The membranes were baked at 80°C for 2 h to immobilize the DNA and incubated in 5% (wt/vol) skimmed milk powder in phosphate-buffered saline plus 0.1% (vol/vol) Tween 20 (Sigma) for 30 min at room temperature. Following three washing steps, the membranes were incubated overnight with the primary antibody H3 at 4°C. After repeated washing, incubation with the secondary antibody (Amersham) was carried out for 2 hours at room temperature. The detection of CPD was done using ECL detection reagents (Amersham) in combination with photosensitive films (Amersham Hyperfilm ECL). Finally, the films were scanned and analyzed using an image analyzer (Gel Doc 2000; Bio-Rad). To quantify the amount of damage in the sample DNA, samples were compared to a calibration series of standard DNA (Promega).

RESULTS

UV-B resistance. The numbers of CFU remaining after 20 min of exposure are shown in Fig. 2 (left panels) for the six studied strains. The corresponding D_{37} values are shown in Table 1. The planktonic *Acinetobacter johnsonii* A2 from Laguna Azul showed the highest survival and the highest D_{37} values. The marine *Pseudomonas putida* strain 2IDINH, the benthic *Cytophaga* sp. strain MF7, and *Acinetobacter johnsonii* ATCC 17909 showed the lowest survival values (in the order of 15 to 20%) and the lowest D_{37} values. The two plankton strains from Laguna Pozuelos showed intermediate survival and D_{37} values. *Pseudomonas* sp. strain MF8 was very close in resistance to *Acinetobacter johnsonii* A2, while *Serratia marcescens* MF42 was less resistant.

Accumulation of DNA photoproducts. The numbers of CPD/Mb accumulated after 20 min of UV-B exposure by each strain are shown in Table 1. The benthic *Cytophaga* sp. strain MF7 was the strain with the largest accumulation of CPD, in accordance with its low survival. In the second place, *Acinetobacter johnsonii* ATCC 17909 accumulated 160 CPD/Mb. However, the pattern for the remaining strains was very different from that described above for resistance. Thus, the most resistant strain, *Acinetobacter johnsonii* A2, was the third in terms

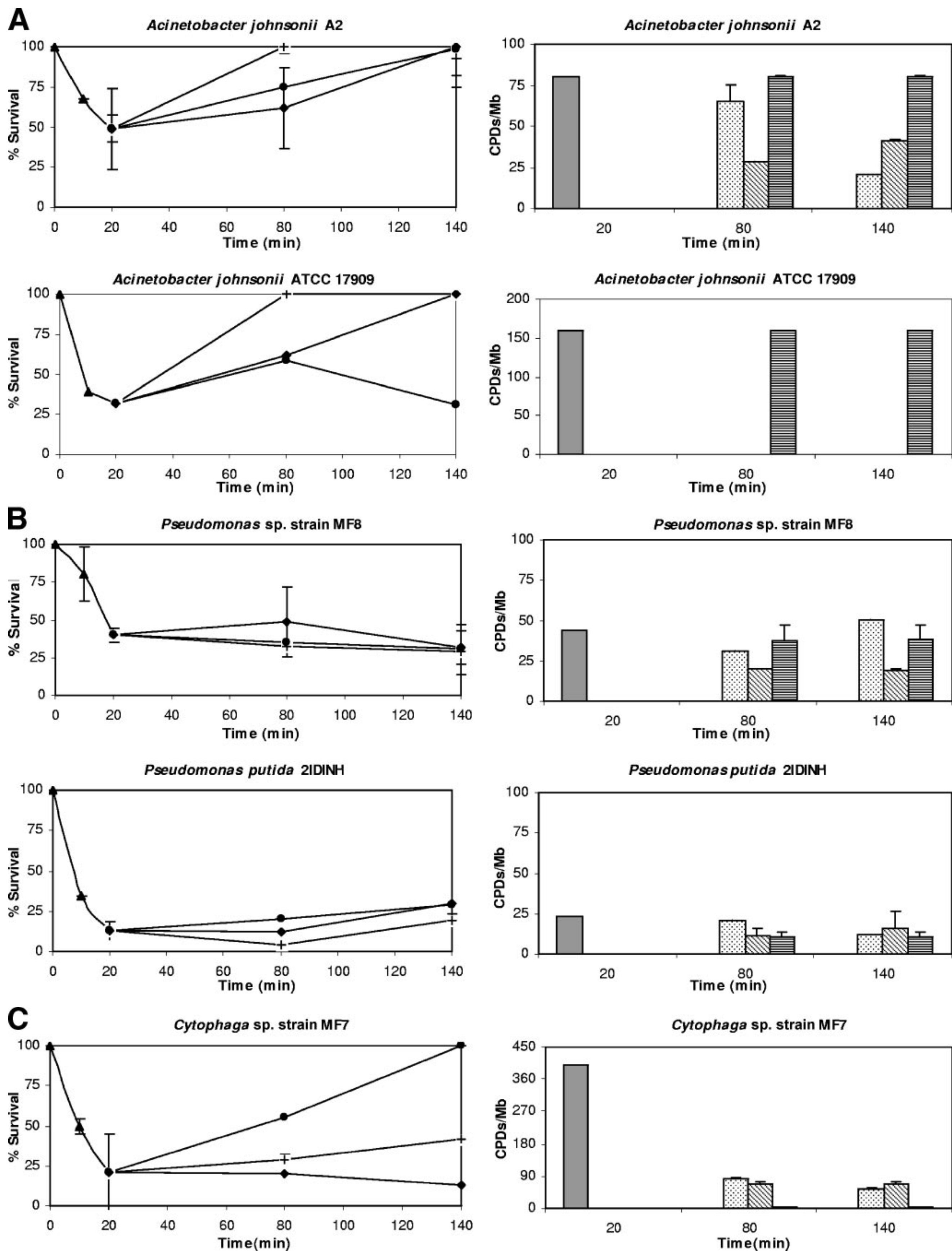

FIG. 2. Effects of UV-B radiation and reactivation on the survival and accumulation of CPD in different bacteria. Lines represent percentages of survival after (▲) UV-B radiation or (+) P, (◆) PA, or (●) DR treatment. Bars represent CPD accumulation after (■) UV-B radiation or (▨) P, (▧) PA, or (▤) DR treatment.

of the number of CPD/Mb, while the least resistant strains, the marine *Pseudomonas putida* strain 2IDINH and *Serratia marcescens* MF42, accumulated smaller amounts of CPD/Mb.

Photoreactivation with PAR alone. All results of repair are shown in Fig. 2 (right panels). The number of CPD/Mb at the end of UV-B exposure has been repeated from Table 1 to facilitate comparison. Both *Acinetobacter johnsonii* strains had very effective photorepair mechanisms, recovering their initial CFU values after 60 min. However, while strain A2 required 120 min to reduce the number of CPD/Mb significantly, in strain ATCC 17909, the number of CPD/Mb was completely reduced during the first 60 min. The two *Pseudomonas* strains were at the opposite extreme, since neither one of them showed any significant recovery or reduction in the number of CPD/Mb after 120 min of incubation. The remaining two strains, *Serratia marcescens* MF42 and *Cytophaga* sp. strain MF7, showed both a limited recovery of the CFU and a limited reduction in the CDP/Mb.

Photoreactivation with PAR plus UV-A. Both *Acinetobacter johnsonii* strains were, again, the most efficient bacteria, showing full recovery of the cell numbers and significant reduction of the numbers of CPD/Mb after 120 min in strain A2 and 60 min in strain ATCC 17909. As was the case with the previous mechanism, the two *Pseudomonas* strains did not show any recovery in cell numbers, even if *Pseudomonas* sp. strain MF8 showed a reduction in the number of CPD/Mb after 120 min. Intriguingly, the remaining strains showed no recovery of CFU (*Cytophaga* sp. strain MF7 and *Pseudomonas* sp. strain MF8) or very limited recovery (*Serratia marcescens* MF42) despite a significant repair of CPD/Mb.

Dark repair. The most resistant strain, *Acinetobacter johnsonii* A2, achieved full recovery of CFU under dark conditions despite its failure to reduce the number of CPD/Mb. In contrast, *Acinetobacter johnsonii* ATCC 17909 did not recover initial CFU values, and neither decreased the number of CPD/Mb. The benthic *Cytophaga* sp. strain MF7 showed not only full recovery of CFU but also an essentially complete reduction of CPD/Mb; it was this unique studied bacterium that presented total recovery (CFU and CPD) with DR. The other three strains did not show any repair under dark conditions.

DISCUSSION

UV-B resistance. In this paper, we show that the UV resistance rates of different isolates were consistent with the isolation environments. In effect, in that way bacteria that were assumed to be exposed to less UV radiation, such as the marine *Pseudomonas putida* strain 2IDINH, the culture collection strain, and the benthic isolate, showed the lowest D_{37} values. In contrast, bacteria isolated from high-altitude wetlands that were assumed to be exposed to higher UV radiation showed higher D_{37} values. Bacteria regarded as tolerant or resistant to UVR have been recovered from different habitats exposed to solar radiation, including aquatic environments, some of them hypersaline, spacecraft assembly, plant phyllosphere, and salt flats (1–3, 9, 11–15, 20, 24, 27, 30, 31). However, some authors found little or no correlation between UVR resistance and natural levels of solar radiation present in the environment (2, 3, 10). In the present study, the four bacteria isolated from environments with high solar radiation showed relatively high UV-B resistance and/or repair abilities compared to those isolated from less irradiated environments. To test this hypothesis and to compare data obtained in our laboratory with those reported in literature, we used a *Pseudomonas* strain and *Acinetobacter johnsonii* ATCC 17909 as external controls. Thus, the marine *Pseudomonas putida* strain 2IDINH was assumed to have a performance comparable to those of marine isolates exposed to analogous treatments in the study by Joux et al. (14) and *Acinetobacter johnsonii* ATCC 17909 as an external control from the same species for strain A2.

Different responses to UV-B inhibition and CPD accumulation were observed in the studied strains. (i) Low survival and high CPD accumulation. Low survival and high CPD accumulation indicated a lack of the photoprotection mechanisms that reduce DNA damage and cell death by UV-B. This was the case for the benthic bacterium *Cytophaga* sp. strain MF7 and *Acinetobacter johnsonii* ATCC 17909.

(ii) Low survival with low CPD formation. In the case of low survival with low CPD formation, the target of damage could be something other than DNA (lipids or proteins), the DNA could be protected by efficient DNA protection machinery, and/or this strain was unable to support DNA damage even though it had been insignificant. This is the case for *Serratia marcescens* MF42 and the marine *Pseudomonas putida* strain 2IDINH.

(iii) High survival with high CPD formation. In the case of high survival with high CPD formation, there could be a DNA lesion bypass, a typical mechanism of the error-prone dark repair that keeps the cell active with DNA replication (by polymerase V) without DNA damage repair. This was the case for *Acinetobacter johnsonii* A2.

(iv) High survival with low CPD formation. In the case of high survival with low CPD formation, we could have efficient photoprotection and photorepair mechanisms as is the case for *Pseudomonas* sp. strain MF8.

Recovery. We found that, independently of the isolation environment, recovery strategies were different among the different bacteria. Thus, *Acinetobacter johnsonii* A2 and *Cytophaga* sp. strain MF7 had the best recovery under most treatments, both in the decrease of CPD and in CFU recovery, reaching the initial CFU values in at least one treatment. Recovery in *Serratia marcescens* MF42 was intermediate, while both *Pseudomonas* spp. demonstrated a low capacity for recovery, with middle recovery in strain MF7 with PA and no recovery at all in the marine *Pseudomonas putida* strain 2IDINH.

***Acinetobacter johnsonii*.** A previous report has shown the effect of solar UV radiation on an *Acinetobacter* sp. isolated from Antarctic marine waters (11). To our knowledge, there are no reports of *Acinetobacter johnsonii* as a UV-resistant bacterium. The *Acinetobacter johnsonii* A2 strain was isolated from Laguna Azul by irradiating total water for 36 h (4.94 W/m^2); together with *Nocardia* sp. strain A5, *Acinetobacter johnsonii* A2 was one of the most resistant strains (8). Moreover, *Acinetobacter johnsonii* A2 was more resistant to UV radiation and accumulated fewer CPD than the reference strain ATCC 17909. However, photorepair mechanisms were efficient in both strains, denoting that this ability could be a phylogenetic characteristic. On the other hand, *Acinetobacter johnsonii* A2, the most resistant strain, accumulated more CPD than the most sensitive strains, excluding the benthic isolate.

These results are intriguing and suggest that this bacterium may have cellular or efficient repair systems, mainly photoprotective mechanisms that work in correlation with UV-B DNA damage. These could allow adaptation to the large amounts of UV-B in the environments. The fact that an efficient photorepair mechanism was also found in the external control (ATCC 17909) would support this idea.

Both *Acinetobacter johnsonii* strains recovered initial CFU values with the three treatments (PA, P, and DR). Light treatments reduced CPD content, but no CPD decrease was found in the dark treatment (Fig. 2). This indicates that in this genus, there is a recovery of cell viability despite the persistence of CPD damage, showing a mechanism that enables lesion bypass during DNA replication. This mechanism allows cells to survive in the presence of unrepaired lesions but is accompanied by an increase in the cellular mutation rates (26). Another explanation could be that low CPD damage (up to 80 or 160 photoproducts/Mb) would be insignificant to trigger the nucleotide excision repair system and lesions would then be repaired by photoreactivation only. This is the case for *Escherichia coli* and many other bacteria, where nucleotide excision repair expression is differentially inducible, depending on the amount of DNA damage experienced by the cells (22, 26). More experiments increasing the amount of photodamage should be done to test this hypothesis.

***Cytophaga* sp.** The benthic *Cytophaga* sp. strain MF7 was also able to recover very well. The number of CPD decreased in all treatments. Maybe benthic bacteria are frequently transported to the photic layer in these shallow wetlands, which are subject to strong winds. Strains from this genus are usually involved in degradation processes in the benthic community of aquatic environments, where penetration of UV-A and UV-B radiation drops very fast depending on the water attenuation coefficient; thus, it is easy to explain that *Cytophaga* sp. strain MF7 was very sensitive to UV-A and UV-B damage and that it was more efficient in repairing its damage and CFU with DR and P treatments. In fact, as was mentioned before, it was the only strain that recovered completely (CFU and CPD) with dark repair.

***Pseudomonas* spp.** *Pseudomonas* spp. are members of the natural bacterial communities in freshwater and nonsaline terrestrial environments. The DNA repair mechanisms of some members of the genus have been well characterized (13–18, 23, 25). Generally, they lack the inducible error-prone DNA repair system (14, 15, 21), which would explain their relatively high UV sensitivity. Results obtained with DR and photorecovery in this study showed that neither strain was very efficient in its recovery after UV-B irradiation.

Independently of the differences in UV resistances between the high-altitude isolates and their lower-altitude controls, photorepair ability in the case of *Acinetobacter* sp. strains and its deficiency in *Pseudomonas* spp. seem to be characteristics inherent to their phylogenetic affiliation. However, more experiments should be performed to support this suggestion.

In summary, we have found that (i) bacteria isolated from high-altitude wetlands showed a range of UV-B resistance and repair capacities consistent with high-mountain habitats and that (ii) the isolates studied showed a diversity of responses to UV-B, showing that bacteria have different strategies to cope with this environmental factor.

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