Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation

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ABSTRACT: The effect of solar ultraviolet radiation (UVR) on viability of natural bacterioplankton assemblages from Antarctic waters, as well as on 2 cultures of isolated bacterial strains (*Acinetobacter* sp. and *Bacillus* sp.), was determined by both *in situ* and temperature-controlled incubator experiments. When natural assemblages were incubated *in situ* at 0.5 m depth, the mean percentage survival fractions (of the bacteria forming colonies on agar) were 13% when the sample was exposed to all UVR, 27% when UV-B radiation was eliminated with a prefilter, and 85% when all UVR was excluded. The magnitude of UVR-induced inhibition decreased with depth so that there was no significant inhibition at 9.5 m. There was very little effect of photosynthetically available radiation (PAR), even at 0.5 m depth. The loss of viability due to UVR or PAR was much greater for the 2 isolated strains than for the natural bacterial assemblages. Exposure of the *Bacillus* sp. to incident PAR, PAR + UV-A, and PAR + UV-A + UV-B resulted in survival values of 9, 0.4, and 0.1%, respectively; when irradiance to the sample was reduced to about 3% of the incident value, the corresponding values were 80, 50, and 24%, respectively. With *Acinetobacter* sp., the corresponding values were 60, 13, and 1.5% with direct exposure to solar radiation, while at 12% of incident radiation no inhibition by either UVR or PAR could be detected . The SOS-repair system could be induced in both bacterial strains studied with the result that the loss in viability due to UVR radiation was much reduced, but both strains still showed some loss of viability when compared to the control samples.

KEY WORDS: Bacterioplankton · Ultraviolet radiation · Antarctica · Viability

INTRODUCTION

It is generally recognized that bacterioplankton biomass in the euphotic zone of marine waters usually amounts to 10 to 50% of the phytoplankton biomass, and that a large fraction of the primary production is either assimilated or respired by the bacterial assemblages (Cho & Azam 1990). Since the progressive deepening of the seasonal ozone hole in the Antarctic during the 1980s, much effort has been devoted to studying the impact of ultraviolet-B (UV-B, 280 to 320 nm) and UV-A (320 to 400 nm) radiation on phyto-

plankton (see Weiler & Penhale 1994). Most of the studies with phytoplankton have shown that UV-A radiation is responsible for a larger decrease in photosynthetic rates than UV-B radiation (Helbling et al. 1992, Smith et al. 1992, Holm-Hansen et al. 1993). Much of the inhibition of photosynthesis in phytoplankton is thought to be due to structural changes and oxidation of pigments associated with the absorption and transfer of energy in photosynthesis (Young et al. 1993). As bacterioplankton have very different cellular absorption spectra as compared to phytoplankton, the relative impacts of UV-A and UV-B radiation on metabolic processes in bacterioplankton cannot be assumed to be similar to phytoplankton, but must be determined by direct experimentation.

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In this paper we report the effect of solar UVR (UV radiation) on the viability of natural Antarctic bacterio-plankton assemblages, as well as on 2 isolated species (*Acinetobacter* sp. and *Bacillus* sp.) when exposed to various photoregimes *in situ* or in temperature-controlled incubators. Also, the importance of the SOS-repair system, a regulatory system that increases the expression of genes for repair of DNA damage, was studied for the 2 isolated bacterial species.

MATERIALS AND METHODS

Our work was performed at Palmer Station, Antarctica (64.7° S, 64.1° W) during November–December 1993. Natural bacterioplankton assemblages were obtained from surface water samples taken with a sterile polycarbonate bottle (1 l) at a station in Arthur Harbor, Antarctica, from the bow of a slowly moving Zodiac boat. These samples were either used directly for the *in situ* experiments or used to isolate individual bacterial strains. Isolation was done by diluting 1 volume of the sample with 2 volumes of isotonic marine broth (Difco 0791-01-2). After 12 h at 4°C, subsamples were streaked onto either nutrient marine agar (Difco 0142-01-8; 1.5%) or Schaedler agar (Difco 0534-17-6; 1.5%) in petri dishes. After incubation for 48 h at 4°C, selected colonies were isolated and identified following the methodology described by Austin (1982). Two isolates (a Gram-negative *Acinetobacter* sp. and a Gram-positive *Bacillus* sp.) were selected for experimental use and were maintained on nutrient marine agar. Before use in any experiment, an inoculum was transferred to marine broth and the suspension incubated for 18 to 24 h in order to reach the exponential growth phase. Aliquots (0.5 ml) of the suspension were then transferred to 50 ml quartz tubes (Teflon-lined screw caps) containing 50 ml of sterile seawater to reach a concentration of 10^5 to 10^6 cells ml^{-1}. When using natural bacterial assemblages in experiments, the 50 ml quartz tubes were filled directly with the water sample.

The experimental samples were exposed to 3 different spectral irradiation regimes: (1) samples in quartz tubes, received UV-B + UV-A + photosynthetically available radiation (PAR, 400 to 700 nm); (2) samples in tubes covered with a Mylar filter, received UV-A + PAR; (3) samples in tubes covered with a Plexiglas (UF3) filter, received only PAR. The Mylar and Plexiglas filters have 50% transmission at 323 and 400 nm, respectively.

Two types of incubations were performed to assess the effects of solar radiation on viability of the bacterial suspensions, as influenced by the spectral irradiance or the fluence of the radiation to which the sample was exposed. (1) For *in situ* incubations, 6 of the 50 ml tubes (duplicates of the 3 different spectral irradiance

regimes) were placed on black-anodized aluminum frames, which were then placed at various depths in the water column between 0.5 to 17 m. (2) The tubes with samples of the 2 isolated bacterial strains were incubated in an outdoor water bath which was exposed to direct sunlight and cooled with flowing seawater. All incubator experiments also included dark control samples which were covered with aluminum foil. In some of these experiments, incident solar radiation was attenuated with various layers of neutral density screening so that irradiance received by the samples varied from 100 to 3% of the incident radiation. Other experiments were designed to assess the importance of the SOS-repair system (Little & Mount 1982, Sancar & Sancar 1988). In these experiments, the samples were incubated with nalidixic acid, which interferes with DNA replication and thus induces the SOS-repair system (Piddock & Walters 1992), at a concentration of 50% of the minimal inhibitory concentration (MIC) for 1 h preceding the experiment. The MIC for each of the 2 bacterial strains had been determined in preliminary tests; the concentrations used for the exposure experiments were 10 mg l^{-1} for *Bacillus* sp. and 20 mg l^{-1} for *Acinetobacter* sp.

Viability of the bacteria was determined by counting colonies on plates at the beginning of each experiment and after the incubations. Plates of Schaedler agar were inoculated with 50 µl of each dilution of the original samples (final dilutions of 10^{-3}, 10^{-4}, and 10^{-5}) and incubated for 48 h. Counting was done on the plates which had between 50 and 250 colony-forming units (CFU).

Incident solar radiation was recorded once per minute, using a spectroradiometer (PUV-510, Biospherical Instruments, Inc.) that measures UVR at 4 wavelengths (305, 320, 340, and 380 nm) and also PAR. The underwater attenuation of solar radiation was measured with a PUV-500 spectroradiometer (Biospherical Instruments, Inc.) that has the same 5 channels mentioned above, in addition to sensors for depth and temperature.

RESULTS

Data from *in situ* incubations performed with natural bacteria assemblages, as well as with the strains of *Acinetobacter* sp. and *Bacillus* sp., are shown in Fig. 1 The viability of natural bacterial assemblages (Fig. 1A) was significantly decreased by UVR, but not by PAR. The survival fraction of samples incubated at 0.5 m in quartz tubes without any covering filter was 0.13 (i.e. the percentage of viable cells was 13% as compared to dark control samples). When UV-B was excluded from the sample, the survival fraction increased to 27%. The magnitude of this UVR-induced inhibition decreased
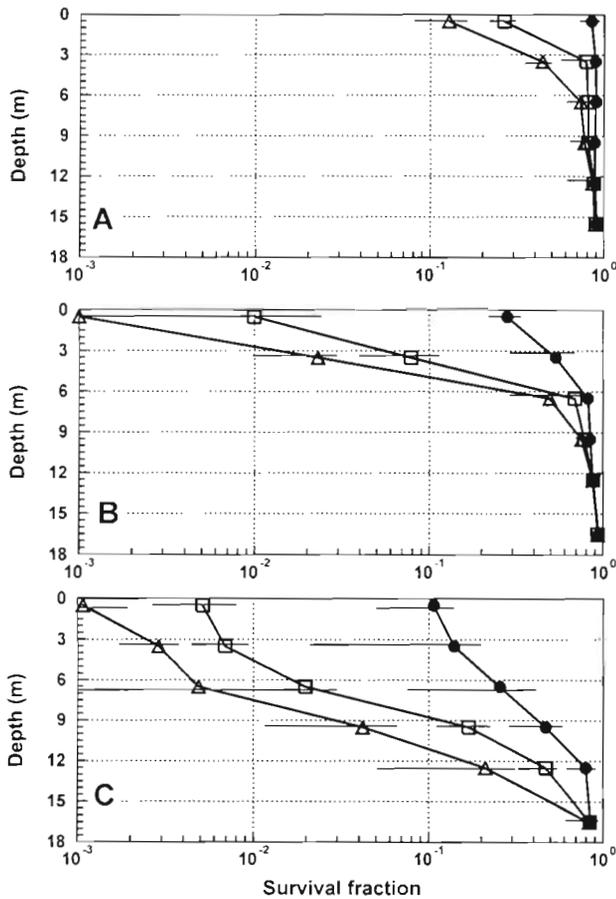
Fig. 1. Data from *in situ* incubations showing mean survival fractions of bacterial cells as a function of incubation depth for 3 different treatments: ($\triangle$) quartz, which received all radiation; ($\square$) Mylar which received UV-A and PAR; and ($\bullet$) Plexiglas UF-3 which received only PAR. Survival fractions are based on numbers of CFU compared to control samples kept in darkness. All samples were incubated for 5 to 7 h during midday. Horizontal lines: 1 SD around the mean. (A) Natural bacterial assemblages incubated on December 16 and 19, receiving a mean dose (at 320 nm) of 2400 J m^{-2} at the sea surface. Mean PAR during the incubation period was 0.062 μE cm^{-2} s^{-1} and mean ozone column concentration at noon was 275 Dobson Units (DU). (B) Strain of *Acinetobacter* sp. incubated on December 10, 13, and 16, receiving a mean dose (at 320 nm) of 2500 J m^{-2} at the sea surface. Mean PAR during the incubation period was 0.078 μE cm^{-2} s^{-1} and mean ozone concentration 290 DU. (C) Strain of *Bacillus* sp. incubated on December 10, 13, and 16, receiving a mean dose (at 320 nm) of 2600 J m^{-2} at the sea surface. Mean PAR during the incubation period was 0.08 μE cm^{-2} s^{-1} and mean ozone concentration 290 DU

exponentially with depth, so that there was no significant inhibition detected at 9.5 m (Fig. 1A). The 2 bacterial isolates were much more sensitive to solar radiation than the natural bacterial assemblages, with *Bacillus* sp. being the more sensitive of the 2 isolates. The viability of both strains was much reduced by both UVR and PAR in surface waters, but there were no sig-

nificant differences among the 3 treatments at 9.5 m depth for *Acinetobacter* sp. (Fig. 1B) and at 16.5 m for *Bacillus* sp. (Fig. 1C). UV-A radiation was responsible for a greater proportion of the loss of viability than UV-B for both bacterial strains.

In order to estimate the relationship between the spectral irradiance incident upon the experimental samples when incubated *in situ* and the extent to which cell viability was decreased, the attenuation of solar radiation in the upper water column was measured during all *in situ* deployments. Data in Fig. 2 show the attenuation of UVR and PAR in the upper 40 m for the days with maximal and minimal penetration of solar radiation in the water column (Fig. 2A, B, respectively). On November 19, when phytoplankton biomass was relatively low (0.6 mg chl *a* m^{-3}) and uniformly distributed in the upper 20 m of the water column, the attenuation coefficients were 0.29, 0.27, 0.21, 0.096, and 0.067 m^{-1} for 305, 320, 340, and 380 nm and PAR, respectively (Fig. 2A). On December 27, the
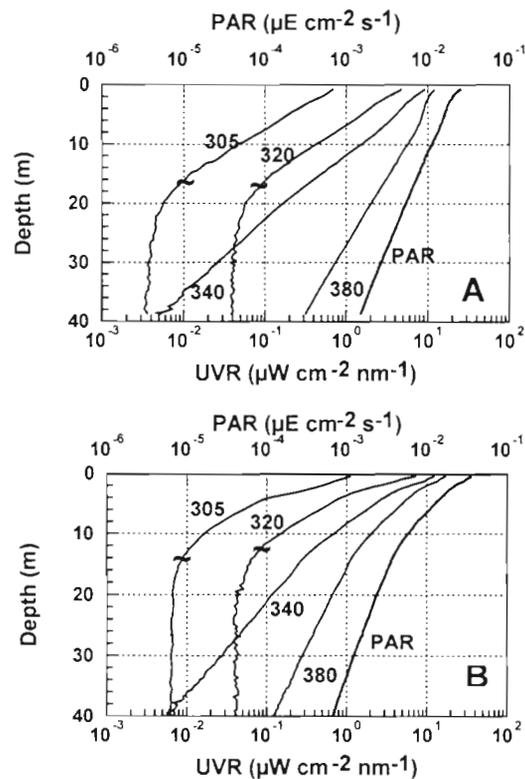

Fig. 2. Underwater radiation field as a function of depth for 4 channels (305, 320, 340, and 380 nm) in the UVR region of the spectrum, and for PAR. 'S' shaped lines on profiles for irradiance at 305 and 320 nm indicate the limits of reliable detection for that sensor. (A) Data obtained on November 19, 1993, when chl *a* concentrations in the upper water column were all close to 0.6 mg m^{-3} (B) Data obtained on December 27, 1993, when the chl *a* concentrations in the upper 12 m of the water column were close to 3.2 mg m^{-3}, and approximately 0.6 mg m^{-3} below that depth

attenuation coefficients were higher (0.46, 0.38, 0.33, 0.21, and 0.16 m^{-1} for 305, 320, 340, and 380 nm and PAR, respectively) in the upper 12 m of the water column (Fig 2B) where the phytoplankton biomass was quite uniform with chl a values of 3.2 mg chl a m^{-3}. Below 12 m, phytoplankton biomass decreased rapidly with depth and this was reflected in much lower attenuation coefficients.

When *Acinetobacter* sp. and *Bacillus* sp. were exposed to varying irradiances in the temperature-controlled incubators, the results were consistent with the *in situ* data in that solar radiation caused a much greater loss of viability in *Bacillus* sp. as compared to *Acinetobacter* sp. (Fig. 3). UV-B, UV-A, and PAR all caused loss of viability in both bacterial strains, but for

Acinetobacter sp. there was a threshold between 0.01 and 0.02 µE cm^{-2} s^{-1} (corresponding to about 1.6 µW cm^{-2} at 320 nm; Fig. 3A), below which there was no loss of viability detected. No such threshold was apparent in the response by *Bacillus* sp., as viability was still reduced when the irradiance of PAR was approximately 0.002 µE cm^{-2} s^{-1} (Fig. 3B). As noted before, the effect of UV-A radiation was greater than that of UV-B radiation in causing loss of viability.

Data from the experiments designed to study the importance of the SOS system in repairing UVR damage are shown in Fig. 4. When the strains were transferred directly from laboratory conditions (see above) and ex-
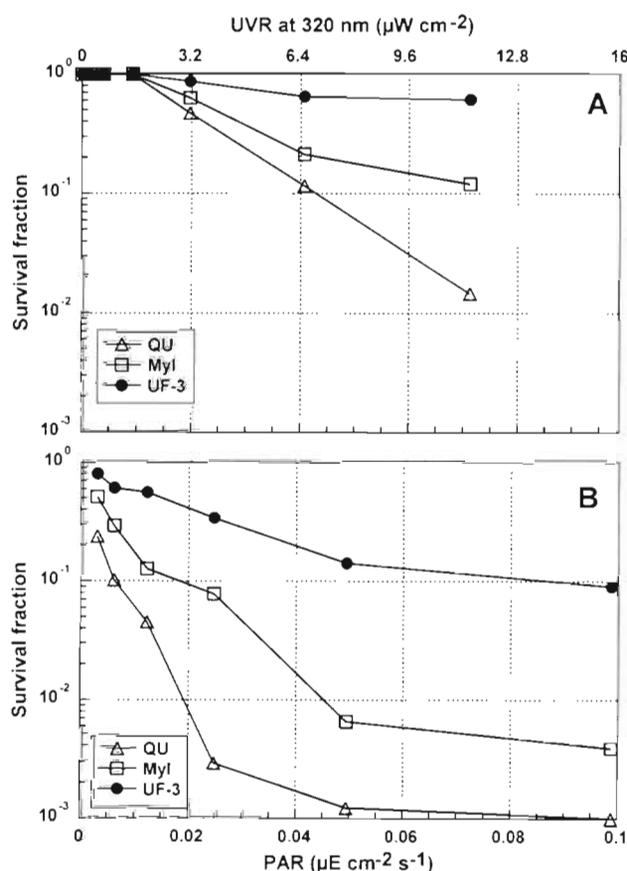

Fig. 3. Survival fraction of 2 bacterial strains as a function of the mean incident irradiation at 320 nm (upper abscissa) and of PAR (lower abscissa) during the incubation period for 3 different treatments: (△) quartz, which received all radiation; (□) Mylar, which received UV-A and PAR; and (●) Plexiglas UF-3, which received only PAR. Survival fractions are based on numbers of CFU compared to control samples kept in darkness. Ozone column concentration at noon was 285 DU. (A) Strain of *Acinetobacter* sp., which received a dose (at 320 nm) of 1500 J m^{-2} for the unattenuated sample. (B) Strain of *Bacillus* sp., which received a dose (at 320 nm) of 2800 J m^{-2} for the unattenuated sample
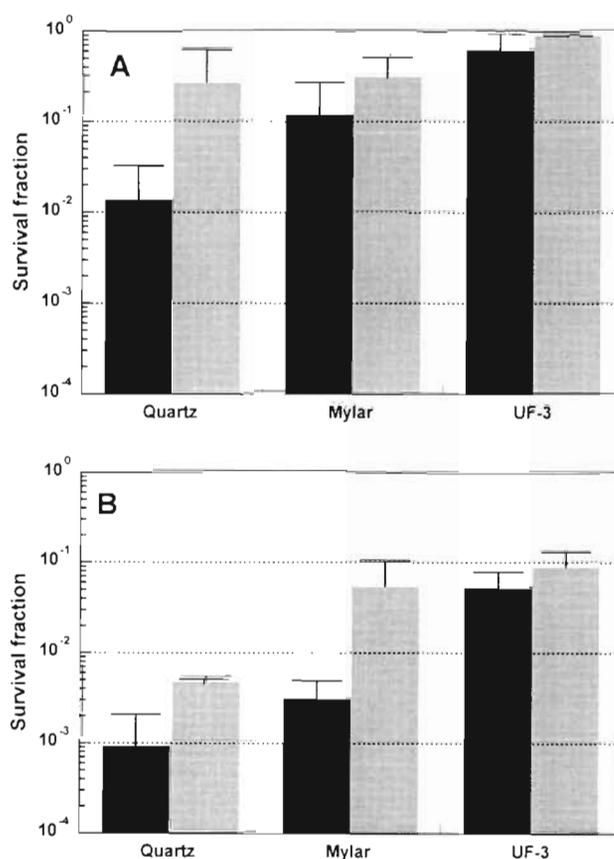

Fig. 4. Survival fraction in 2 bacterial strains before and after induction of the SOS-repair system (dark and light bars, respectively) and when exposed to 3 different radiation regimes: quartz, which received all radiation; Mylar, which received UV-A and PAR; and Plexiglas UF-3, which received only PAR. Survival fractions are based on numbers of CFU compared to control samples kept in darkness. Vertical lines on top of the bars indicate 1 SD. (A) Strain of *Acinetobacter* sp. incubated on December 11 and 22 for 5 to 6 h, receiving a mean dose (at 320 nm) of 2200 J m^{-2}. Mean PAR during the incubation period was 0.08 µE cm^{-2} s^{-1} and mean ozone concentration 290 DU. (B) Strain of *Bacillus* sp. incubated on December 11, 16 and 22 for 5 to 6 h, receiving a mean dose (at 320 nm) of 2400 J m^{-2}. Mean PAR during the incubation period was 0.11 µE cm^{-2} s^{-1} and mean ozone concentration 295 DU

posed to solar radiation, they showed a decrease in the survival fraction, especially in the treatments receiving all UVR. As noted previously, *Bacillus* sp. also showed a greater loss of viability due to high irradiance of PAR (Fig. 4B) than did *Acinetobacter* sp. (Fig. 4A). However, when the SOS-repair system was activated before exposure to solar radiation, the survival fraction was significantly higher in all samples receiving UVR, as compared to the samples which did not have the SOS system induced. With the SOS-repair system induced, no significant differences were observed between the quartz and Mylar treatments for *Acinetobacter* sp., but the viability was still less than in the samples that were not exposed to any UVR. Although *Bacillus* sp. showed higher viability when the SOS-repair system was induced, viability in the samples receiving UV-B was still significantly lower than when UV-B was removed by the Mylar filter (Fig. 4B).

DISCUSSION

The data presented above for natural assemblages of bacterioplankton, as well as for the 2 isolated bacterial strains, indicate that viability of bacteria in Antarctic waters may be markedly decreased by exposure to solar UVR. The impact of UV-A on cell viability was consistently greater than the impact of UV-B radiation. If the loss of cell viability is due to DNA damage resulting from UVR, one would expect that UV-B wavelengths, which overlap the absorption curve for DNA, would be the primary spectral region responsible for loss of viability. The fact that UV-A wavelengths seem to be more effective than UV-B radiation in regard to loss of cell viability suggests that damage to the DNA replication process may be via photodynamic reactions involving active oxygen species rather than by direct absorption of UVR by DNA. This interpretation would be similar to that discussed by Setlow et al. (1993) for their experimental results involving the effects of UV-A radiation on the initiation of melanomas in fish.

It should be noted that there were considerable differences in sensitivity to UVR and PAR of the 2 strains of bacteria isolated from Antarctic waters as compared to that of the natural bacterioplankton assemblages. The experimental results with the isolated strains should thus be viewed as indicative of a small fraction of the natural bacterial assemblages and cannot be extrapolated reliably to predict possible effects of enhanced UVR on the marine ecosystem. As only a few percent of marine bacteria generally will form colonies when spread onto agar plates, any bacterial isolates obtained from agar plates may not be very representative of the natural bacterial assemblages. However,

results from such isolates can be useful in studying specific cellular responses to environmental stress, such as the ability to induce DNA repair processes.

Although the viability of the natural bacterial assemblages was only slightly affected by PAR, the viability of both bacterial isolates was much reduced by such visible radiation. The threshold value for inhibition of viability by PAR in *Acinetobacter* sp. was between 0.01 and 0.02 μE cm^{-2} s^{-1}, which would correspond to a depth of approximately 15 m on a sunny day for the water column shown in Fig. 2A. For *Bacillus* sp., the lowest irradiance of PAR used in our experiments (approximately 0.002 μE cm^{-2} s^{-1}, equivalent to about 0.32 μW cm^{-2} at 320 nm) was still inhibitory in regard to cell viability; this irradiance would correspond to a depth of about 40 m during the time of our experiments. These threshold irradiance values are lower than the threshold value of 3.1 μW cm^{-2} at 320 nm for inhibition of photosynthesis in Antarctic phytoplankton as reported by Helbling et al. (1994). It thus seems that viability of bacteria in Antarctic waters may be more sensitive to solar UVR than inhibition of phytoplankton primary production. Our *in situ* data on natural bacterioplankton assemblages are comparable with the results of Herndl et al. (1993), who reported a loss of bacterioplankton activity of about 50% in the upper 5 m of the water column. Inhibitory effects on bacterial metabolism by such low irradiances of solar radiation have been recognized for many years in regard to nitrifying bacteria, and have been documented in Antarctic waters by Olson (1981), who reported inhibition of the oxidation of ammonia and of nitrite by solar irradiances less than 1% of incident radiation. As the summer irradiance of solar PAR incident on most Antarctic waters is close to 0.1 μE cm^{-2} s^{-1}, the 1% figure cited by Olson would equate to about 0.001 μE cm^{-2} s^{-1}, which would be a little lower than the lowest irradiance used in our experiments with isolated cultures.

When the 2 strains of bacteria used in our experiments were incubated *in situ*, loss of viability due to solar radiation was much greater than in the natural bacterioplankton assemblages. However, when the SOS-repair system had been induced, loss of viability in *Acinetobacter* sp. due to UVR was comparable with the inhibition recorded for the natural bacterial assemblages; viability was also increased in *Bacillus* sp., but the survival fraction was still considerably lower than that for the natural assemblages. It is thus possible that the sensitivity of the 2 isolated strains to UVR was due to the fact that they had been isolated and grown in the dark in laboratory incubators (i.e. without any UVR stress) and might not have been adapted to solar radiation as were the natural assemblages of bacteria used in our experiments.

In spite of considerable sensitivity of Antarctic bacteria to UVR, the danger of enhanced UV-B radiation resulting from stratospheric ozone depletion on the metabolic activity of bacteria in Antarctic waters would not seem to be as great as that for phytoplankton. In contrast to photoautotrophic cells, which must remain in the euphotic zone in order to maintain net growth rates, bacterial cells need not reside in surface waters to have adequate sources of organic substrates. Although viability of bacteria can be seriously impacted by solar UVR in the upper 10 m of the water column, the euphotic zone in Antarctic waters is generally close to 70 to 90 m (Tilzer et al. 1985, Helbling et al. 1994), so bacteria should not be greatly affected by UVR throughout most of the euphotic zone. Furthermore, it is apparent that bacteria also possess the capacity to acquire mechanisms to repair damage to DNA induced by UVR. Another protecting mechanism which may be operative is the synthesis of UV-absorbing compounds, which apparently do decrease cellular damage induced by UVR in many eucaryotic and procaryotic organisms (Dunlap et al. 1986, Tuveson et al. 1988, Karentz et al. 1991, Garcia-Pichel & Castenholz 1993, Garcia-Pichel et al. 1993), but this has not yet been studied for heterotrophic bacteria in Antarctic waters. Our data suggest that the effects of solar UVR on bacterioplankton in Antarctic waters should receive further attention, especially in regard to the mechanisms of UV-B induced damage, the degree to which cells can adapt to increased fluences of UVR, and to effects of vertical mixing processes in the upper water column.

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