Effect of solar radiation and the subsequent dark periods on two newly isolated and characterized Antarctic marine bacteria

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Two strains of psychrotolerant Antarctic marine bacteria were isolated and characterized using biochemical and molecular techniques. Sequencing of 16S rRNA gene showed that UVvi strain belongs to the genus *Arthrobacter* whereas UVps strain is related to the *Flexibacter–Cytophaga–Bacteroides* (FCB) group. Response of the strains to solar radiation was studied during the summer of 1999 in Potter Cove, near Jubany station (South Shetland Island, Antarctica). The effect of photosynthetically available radiation (PAR, 400 - 700 nm), ultraviolet-A (UV-A, 320 - 400 nm) and ultraviolet-B radiation (UV-B, 280 - 320 nm) on cell viability was studied using mixed cultures in quartz bottles covered with interferential filters and exposed to solar radiation. In all experiments, four treatments were used: dark (with light screened out), PAR (with UV radiation screened out), PAR + UV-A (UV-B screened out) and PAR + UV-A + UV-B. Under the assayed conditions, PAR + UV-A and PAR + UV-A + UV-B radiation showed similar negative effects on the viability of the studied strains. However, at the end of the exposure time, mortality values in PAR + UV-A + UV-B treatments were higher than those observed under PAR + UV-A treatments. In both PAR + UV-A and PAR + UV-A + UV-B treatments we observed high levels of hydrogen peroxide compared with the dark control. The *Arthrobacter* UVvi strain showed significant recovery in dark conditions after exposure to the PAR + UV-A but not after the PAR + UV-A + UV-B treatment. This strain proved to be more resistant to UV radiation than the FCB group-related UVps strain. The results showed that UV radiation has a deleterious effect on these Antarctic marine bacteria and also revealed that the analysed components of the Antarctic bacterioplankton may have different responses when they are exposed to the same irradiance conditions.

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The cyclic annual depletion of the stratospheric ozone layer over Antarctica and the Southern Ocean during spring is common knowledge (Staehelin et al. 2001). This decrease in the ozone concentration selectively reduces stratospheric absorption of ultraviolet-B radiation (UV-B, 280 - 320 nm), resulting in higher irradiance on the Antarctic Earth's surface (Lubin et

al. 1989). As reported by many authors, the UV-B increment is responsible for significant biological effects on diverse aquatic environments (Aas et al. 1996; Herndl et al. 2000; Vincent & Neale 2000) and can affect the species composition of aquatic microbial communities (Davidson 1996). However, the majority of the studies dealing with the effect of UV on aquatic organisms have been focused on phytoplankton assemblages, the effect on the aquatic bacteria being much less investigated although they play a key role in mineralization of nutrients and provide a trophic link to higher organisms (Azam et al. 1983; Ducklow et al. 1992). This role is crucial in the Southern Ocean where phytoplankton stocks are low and the spring algal blooms do not develop (Karl 1993). A number of studies showed that UV-B radiation is directly harmful for marine bacteria (Herndl et al. 1993; Miller et al. 1999), which seem to be more susceptible to the detrimental effect of UV radiation than other planktonic organisms (Jeffrey et al. 1996). The apparent absence of UV protective compounds in marine bacteria other than cyanobacteria could be one of the major causes of this susceptibility (Cockell & Knowland 1999). Species composition of marine bacterial communities is also affected by UV-B stress as was reported by Arrieta et al. (2000). In addition to the UV-B effect, ultraviolet A radiation (UV-A, 320 - 400 nm) may be responsible for certain types of photobiological damage (Kim & Watanabe 1994; Sommaruga et al. 1997) affecting bacterial viability and activity (Helbling et al. 1995) either directly or indirectly via the production of oxidant compounds from dissolved organic matter such as hydrogen peroxide.

The effects of solar radiation on bacterioplankton are not exclusively detrimental but UVA and PAR also play crucial roles in the repair of DNA damage as they activate photoenzymatic repair mechanisms (Kaiser & Herndl 1997). This fact determines a complex relationship between the positive and negative effects of solar UV radiation on these organisms, which largely depends on the local characteristics of the environments under study. Thus, the analysis of the response of autochthonous bacterial strains is essential to understand how solar radiation affects the dynamic of the bacterial community of one particular environment.

In this study two culturable Antarctic marine bacteria, which proved to be dominant in the agar plate cultures, were isolated from surface seawater for the following purposes: to characterize them using biochemical and molecular tests; and to analyse the recovery capacity of the strains during dark periods after UV-B, UV-A and PAR exposure.

Materials and methods

Study area

Both strains were isolated from surface waters of Potter Cove, a small fjord-like environment located on King George Island (62°14' S, 58°40' W), South Shetland Islands, Antarctica. Field experiments were carried out by direct exposure of the cultured bacteria to solar radiation on a beach in Potter Peninsula. Laboratory experiments were conducted in the Argentinean–German Dallman Laboratory (Jubany Station, Argentina).

Bacterial strains.

Water samples were collected with sterile flasks, streaked on marine nutrient agar plates and incubated at 4 °C. Two bacterial strains were isolated and purified by re-streaking twice. Strains were tested for gram reaction, mobility (phase contrast microscope), oxidase production and glucose oxidation–fermentation test (Hugh & Leifson 1953). Owing to the difficulties in the characterization of environmental bacterial isolates, identification was carried out in two different ways: (1) testing morphological and physiological characteristics by means of the API20 (BioMerieux) and additional biochemical tests and (2) sequencing the 5' end of the 16S rDNA. The selected strains were designated as UVvi (a yellow pigmented aerobic gram negative rod) and UVps (an orange pigmented pleomorphic aerobic rod with a variable response to gram staining). 16S rDNA was isolated from bacterial colonies using standard techniques (Sambrook et al. 1989) and amplified by PCR. Primers used for the amplification corresponded to *E. coli* positions 005 and 531. PCR products were purified using Microcon 100 (Amicon) molecular weight cut-off membranes and quantified by running in agarose gels (1 %). Sequencing of PCR products was carried out by MIDI Labs (Newark, NJ) using an ABI Prism 377 DNA sequencer. The analysis of sequences was performed using PE Applied Biosystem's MicroseqTM microbial analysis software and data base.

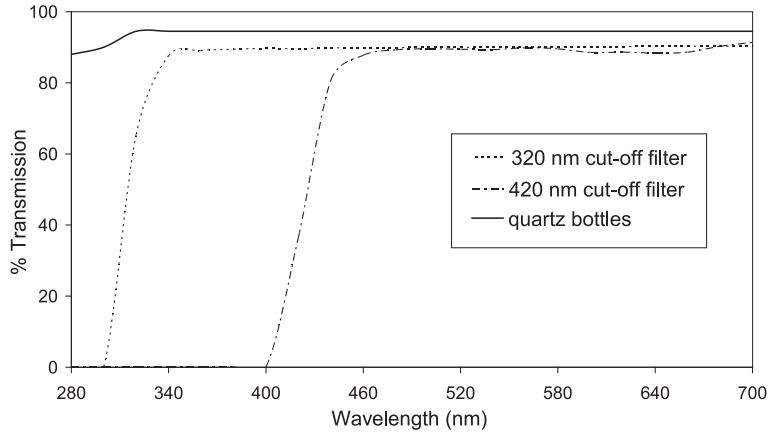

Fig. 1. Wavelength cut-off characteristics of the interferential quartz filters (Schott R) and the quartz bottles used to perform the different treatments of Exp1 and Exp2.

Phylogenetic trees were constructed with the top ten alignment matches using the neighbour-joining method (Saitou & Nei 1987).

Irradiance measurements

Incident solar radiation was measured continuously using a multichannel UV spectroradiometer developed at the Alfred Wegener Institute for Polar and Marine Research and distributed by Isitec Bremerhaven, Germany. This instrument is based on a Bentham DM 150 double monochromator (Bentham), with a multichannel detector system. Data were recorded every second from 290 nm to 320 nm with a data point every 1.35 nm through the 32 detection channels. Data were stored as 1-min average values. The 290 - 320 nm range was used because at wavelengths lower than 290 nm, radiation is below detection limit for the solar altitudes reached at Potter Peninsula.

Evaluation of the solar radiation effect

Two experimental approaches were used. In the first experimental design (Exp1), which was performed on 9 February 1999, the aim was to observe the effects of different solar radiation doses on the isolated strains. For this purpose, two sets of cultures were exposed during different time periods to solar radiation, receiving therefore different accumulated radiation doses. In this assay, concentrated bacterial stock solutions (approximately 10^8 colony forming units [CFU] ml^{-1}) were prepared from marine agar plate cultures. Aliquots of each bacterial stock solutions were mixed and diluted 1:100 in seawater. Two sets of quartz bottles containing 50 ml of the mixed bacterial suspension were prepared and placed in an incubation chamber. This chamber was immersed in a continuous water circulation bath in order to minimize thermal oscillations in the flasks (average temperature during the assay was 5.5 °C ± 1 °C). The first set of flasks (G1) was exposed to solar radiation during 8 h (11:00 to 19:00, local time) and the second one (G2) was exposed only during 5 h but starting 3 h later (14:00 to 19:00). Interferential quartz filters (Schott R) were used to cover the flasks to determine four different irradiance treatments (by triplicate). Treatments considered were: (a) dark control (blackfilter); (b) PAR (420 nm cut-off filter); (c) PAR + UV-A radiation, (320 nm cut-off filter); and (d) PAR + UV-A + UV-B, i.e. the full radiation spectrum available (quartz bottles without filters). Figure 1 shows the spectral distribution of the different filters as well as that of the quartz bottles. Samples from each treatment were taken at different times. Serial dilutions of the samples were plated (0.1 ml) on nutrient marine agar and incubated in the dark for 7 d at 20 °C. This temperature was selected to shorten the incubation period as it is known that the same results are obtained using lower temperatures with these bacteria and with other psychrotolerant bacterial strains (Delille et al. 1988). Counting was done on the plates that presented 30 - 300 colonies and results were expressed as CFU ml^{-1}.

In the second experiment, the aim was to analyse the potential recovery of the bacterial strains during a dark period after exposure to solar radiation. The experimental design was performed using the same mixed culture and conditions

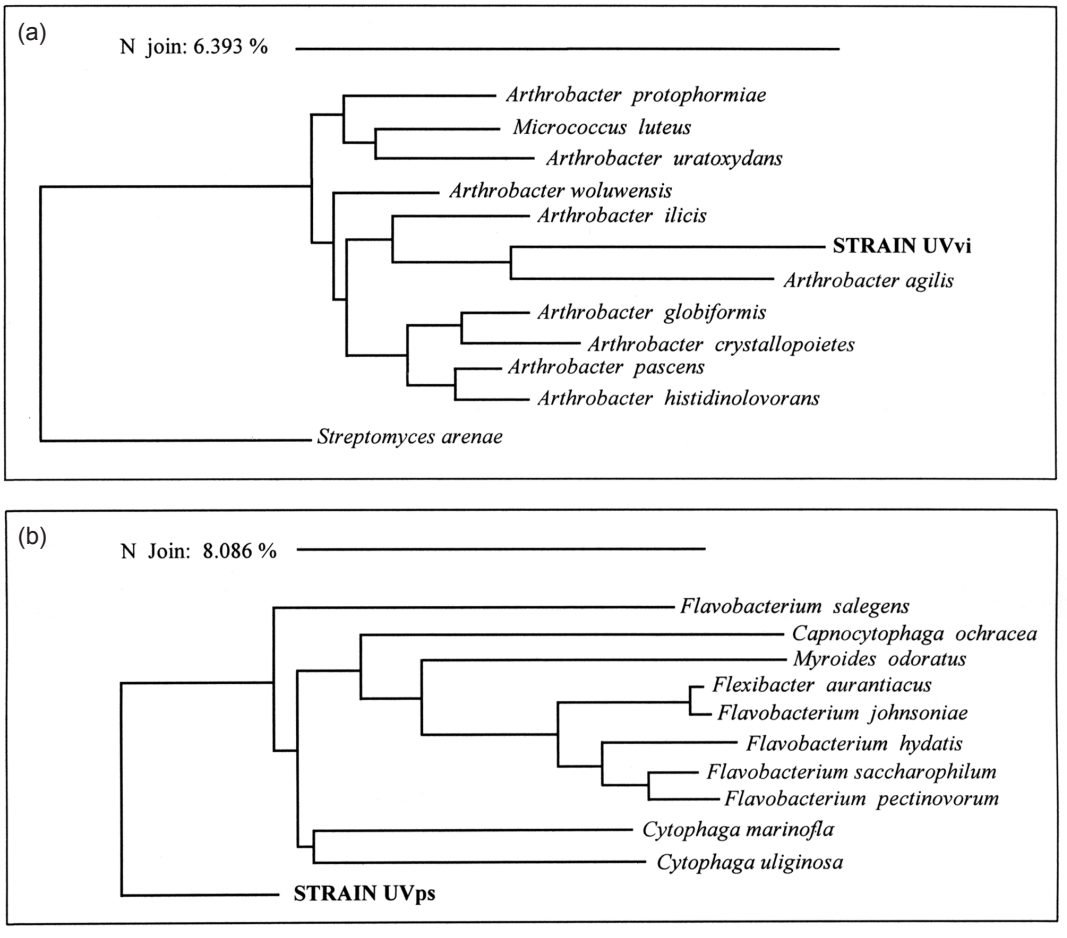

Fig. 2. Neighbour-joining phylogenetic trees showing the relationship of the (a) *Arthrobacter* UVvi and (b) FCB-related UVps strains to the top ten alignment matches obtained using PE Applied Biosystem's Microseq microbial analysis software and data base.

described for the first one but in this case bacteria were kept in the dark after the light exposure period. Two experiments (Exp2a and 2b), differing in the length of the dark period, were carried out. In Exp2a (performed on 13 February 1999), the mixed culture was exposed to solar radiation between 16:00 and 19:30 local time, and was followed by a dark period of 12 h in length. In Exp2b (performed on 22 February 1999), the exposure period was extended between 13:00 and 19:00, local time, and was followed by a dark period of 24 h in length. Bacterial viability was determined as described for the first experimental design. In all cases, concentration of H_2O_2 in water samples taken from the treatments at different times was measured using the photometric method of Bader et al. (1988).

Statistical analysis

Bacterial counts data obtained under different treatments were analysed by repeated measurements ANOVA and Tukey's multiple comparison test. Individual comparisons of pairs of values at the end of the assays were made using Unpaired T-test.

Results

Neither UVps nor UVvi strains could be identified at the genus level using morphological and

physiological tests of API20 Systems. Comparison of the 5' end sequence (500 bp) of the 16S rDNA of UVvi strain with the sequences from the MicroSeq data base showed a percentage of difference of 6.81 with *Arthrobacter agilis* and nine of the top ten closest matches belong to the *Arthrobacter* genus (Fig. 2a). The same result at genus level (but not at species level) was obtained with the Ribosomal Database Project (similarity rank of 0.77 with *A. globiformis*) and with the GenBank (98% DNA sequence identity with *Arthrobacter psychrolactophilus*). On the basis of these data, the strain will be referred to as *Arthrobacter* UVvi. On the other hand, the UVps strain seems to be related to the *Flavobacterium–Cytophaga–Bacteroides* (FCB) group and showed a percentage of difference of 12.21 with *Flavobacterium saccharophilum* (the closest match). Nevertheless, this genetic distance indicates that the UVps strain did not group well within any particular genus present in the MicroSeq data base (Fig. 2b). Comparison using the GenBank data base confirmed that this strain is related to the FCB group (90% DNA sequence identity with *Psychroserpens burtonensis*) but percentage of identity was insufficient to assign this strain to one of the genera present in the consulted data base. The RDP data base did not show a similarity rank indicating genus match either (similarity rank of 0.62 with the marine snow associated clon agg13 was the closest match). On the basis of these data, the strain will be referred to as FCB-related UVps. Partial sequences from the 5' end of 16S RNA gene of *Arthrobacter* UVvi and FCB-related UVps strain were submitted to GenBank and registered under the accession numbers AY220354 and AY220353, respectively.

A significant ($p<0.05$) decrease in CFU was found in the PAR+UV-A and PAR+UV-A+UV-B treatments compared to the dark and PAR controls in the first experiment (Fig. 3). No significant differences were observed either between PAR and dark, or between PAR+UV-A and PAR+UV-A+UV-B treatments. The same pattern was observed for the two studied strains and for the two experimental sets (G1 and G2). However, when only the final values were compared, both strains showed significant differences ($p<0.05$) in CFU ml^{-1} between PAR+UV-A and PAR+UV-A+UV-B treatments for the G1 set but only the FCB-related UVps strain showed significant differences ($p<0.05$) between PAR+UV-A and PAR+UV-A+UV-B treatments for the G2 set (Table 1). In addition, when the count values for both strains were expressed as a PAR+UV-A/PAR+UV-A+UV-B ratio, a continuous increase was observed with time under both G1 and G2 experimental conditions. This pattern suggests a greater susceptibility of FCB-related UVps strain to PAR+UV-A+UV-B radiation compared with *Arthrobacter* UVvi.

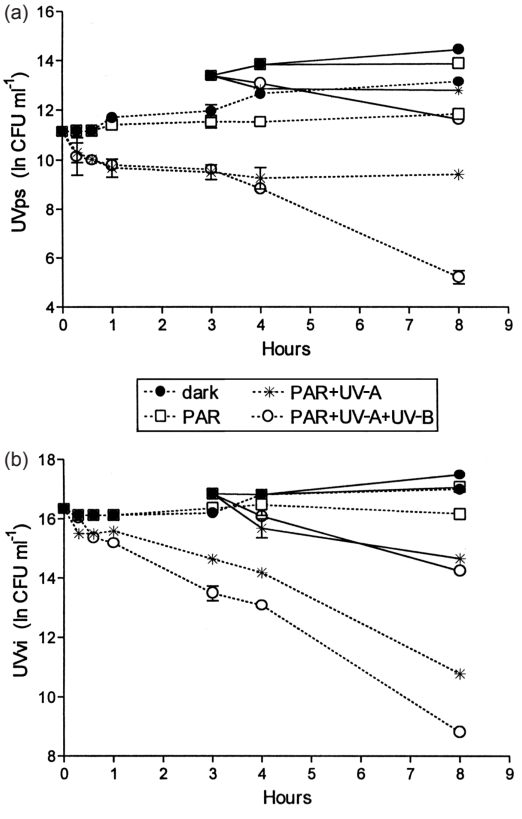

Fig. 3. Effect of solar radiation registered on 9 February 1999 in Jubany Station on viability of (a) FCB-related UVps and (b) *Arthrobacter* UVvi. For both strains two sets of flasks are shown: experimental set G1 (dotted lines), which flasks were exposed to solar radiation during 8 h (11:00 - 19:00 local time) and experimental set G2 (continuous lines), which flasks were irradiated during 5 h, starting 3 h later than G1 set (14:00 - 19:00 local time). Each set of flasks included dark (filled circles), PAR (open quadrants), PAR+UV-A (stars) and PAR+UV-A+UV-B (open circles) treatments. Error bars indicate standard deviation of triplicates.

In the second experimental design, Exp2a and Exp2b showed similar results in relation to the effect produced by the UV radiation treatments when compared with Exp1. Both PAR+UV-A and PAR+UV-A+UV-B treatments caused a major

Table 1. Colony forming units (CFU) of FCB-related UVps and *Arthrobacter* UVvi strains observed at several time of exposure under PAR+UV-A+UV-B (total solar radiation) and PAR+UV-A (solar radiation with UV-B screened out) treatments. G1 and G2 are different experimental groups that were exposed to solar radiation during different irradiation periods (see Materials and methods section for details). PAR+UV-A/PAR+UV-A +UV-B ratios express the relationship between counts under both treatments.

Strain	Experimental group	Time	PAR+UV-A+UV-B (CFU ml^{-1})	PAR+UV-A (CFU ml^{-1})	P ($\alpha = 0.05$)	PAR+UV-A/ PAR+UV-A +UV-B ratio
UVps	G1	1 h	$1.7\ 10^4 \pm 5.9\ 10^3$	$1.4\ 10^4 \pm 2.3\ 10^3$	0.86	0.93
		4 h	$6.9\ 10^3 \pm 1.0\ 10^2$	$1.2\ 10^4 \pm 4.7\ 10^3$	0.43	1.74
		8 h	$1.9\ 10^2 \pm 5.0\ 10^1$	$1.2\ 10^4 \pm 1.2\ 10^3$	0.0095*	65.26
	G2	1 h	$4.8\ 10^5 \pm 8.0\ 10^4$	$3.8\ 10^5 \pm 2.0\ 10^4$	0.35	0.79
		5 h	$1.1\ 105 \pm 7.0\ 10^3$	$3.6\ 10^5 \pm 2.0\ 10^4$	0.0073*	3.19
UVvi	G1	1 h	$3.9\ 10^6 \pm 4.4\ 10^5$	$5.9\ 10^6 \pm 5.7\ 10^4$	0.11	1.49
		4 h	$4.8\ 10^5 \pm 4.0\ 10^4$	$1.4\ 10^6 \pm 6.0\ 10^5$	0.0056*	3.00
		8 h	$4.8\ 10^4 \pm 1.5\ 10^3$	$6.7\ 10^3 \pm 7.0\ 10^2$	0.0016*	7.16
	G2	1 h	$9.7\ 10^6 \pm 1.0\ 10^5$	$6.8\ 10^6 \pm 2.1\ 10^6$	0.30	0.70
		5 h	$1.5\ 10^6 \pm 2.0\ 10^5$	$2.3\ 10^6 \pm 2.1\ 10^5$	0.11	1.51

* Significant differences between CFUs under PAR+UV-A+UV-B and PAR+UV-A treatments.

loss of viability during the solar radiation exposure ($p < 0.05$) when compared with dark and PAR controls (Figs. 4, 5). However, at the end of the irradiation periods of Exp2a and 2b, no significant differences between the UV radiation treatments were observed for *Arthrobacter* UVvi and only in Exp2b was a significant difference ($p < 0.05$) observed between PAR+UV-A and PAR+UV-A+UV-B treatments for FCB-related UVps strain. In Exp2a, mortality values after 1 h exposure under UV-B treatment were 61 % and 13 % for FCB-related UVps and *Arthrobacter* UVvi, respectively. The accumulated dose for such period was 2.12 kJ m^{-2} (exposure between 16:30 and 17:30). In Exp2b, a higher UV-B dose than those registered in the Exp2a (6.03 kJ m^{-2} between 13:00 and 14:00) caused 91 % mortality in FCB-related UVps and 69 % in *Arthrobacter* UVvi. These results confirm the higher susceptibility of FCB-related UVps to PAR+UV-A+UV-B radiation compared with *Arthrobacter* UVvi. Mortality values for both strains at different UV-B doses (Table 2) show that the higher susceptibility of the FCB-related UVps mentioned above is evident at lower UV-B doses. For the highest UV-B doses (higher than approximately 4.3 kJ m^{-2}), magnitude of the damage produced on both strains seems to mask any interspecific differences.

When the effect of the dark period after solar exposure was analysed, FCB-related UVps showed no recovery in counts values under PAR+UV-A and PAR+UV-A+UV-B treatments after 12 and 24 h in darkness. After 12 h in darkness (Fig. 4), FCB-related UVps showed 0.1 % and 4 % of the viable counts observed at the start of dark period under PAR+UV-A+UV-B and PAR+UV-A treatments, respectively. After 24 h in darkness (Fig. 5), FCB-related UVps from the PAR+UV-A+UV-B treatment showed no viable counts, and only 2 % viability under the PAR+UV-A treatment. *Arthrobacter* UVvi showed a different pattern. After 12 h in darkness (Fig. 4) this strain retained 28 % and 83 % of the viable counts present at the start of the dark period in the PAR+UV-A+UV-B and PAR+UV-A treat-

Table 2. Percentages of mortality showed by FCB-related UVps and *Arthrobacter* UVvi strains under different UV-B accumulated doses.

UV-B dose (J m^{-2})	Mortality under PAR+UV-A+UV-B treatment (%)	
	UVps strain	UVvi strain
1218	52	25*
2122	60	12*
2331	68	51*
3258	87	60*
4306	74	68
6030	91	68*
7001	92	92
9828	99	93
12404	90	96
16697	99	99

* Significant differences ($p < 0.05$) in mortality values between FCB-related UVps and *Arthrobacter* UVvi strains.

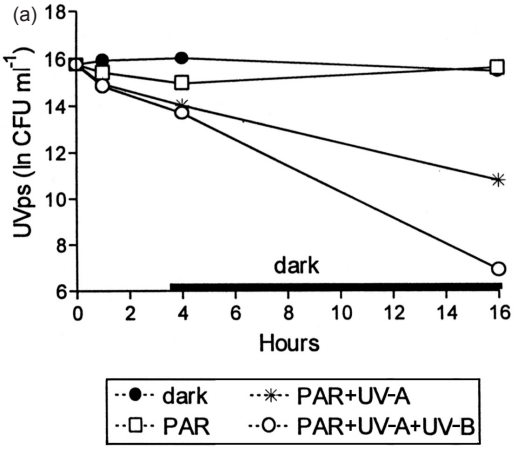

Fig. 4. Effect of solar radiation registered on 13 February 1999 (Exp2a) in Jubany Station on viability of (a) FCB-related UVps and (b) *Arthrobacter* UVvi under dark (filled circles), PAR (open quadrants), PAR+UV-A (stars) and PAR+UV-A+UV-B (open circles) treatments. Flasks were irradiated during 3.5 h (16:00-19:30 local time) followed by a 12 h long dark period. Error bars indicate standard deviation of triplicates.

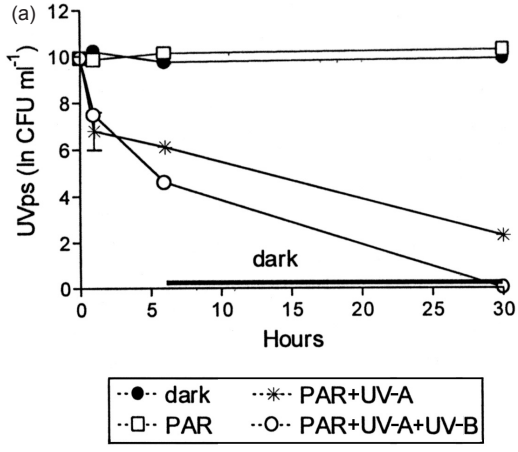

Fig. 5. Effect of solar radiation registered on 22 February 1999 (Exp2b) in Jubany Station on viability of (a) FCB-related UVps and (b) *Arthrobacter* UVvi under dark (filled circles), PAR (open quadrants), PAR+UV-A (stars) and PAR+ UV-A+UV-B (open circles) treatments. Flasks were irradiated during 6 h (13:00-19:00 local time) followed by a 24 h long dark period. Error bars indicate standard deviation of triplicates.

ments, respectively. On the other hand, after 24 h in darkness (Fig. 5), *Arthrobacter* UVvi from the PAR+UV-A+UV-B treatment showed a non-significant increase in CFU ml^{-1} (6%) whereas a significant increase ($p<0.05$) of 45% was observed in counts from the PAR+UV-A treatment.

Figure 6 shows the evolution of H_2O_2 during Exp2b. After 6 h of solar exposure, 64.5 μM and 35.6 μM H_2O_2 from the PAR+UV-A+UV-B and PAR+UV-A treatments, respectively, were observed. These values were significantly higher ($p<0.05$) than those observed for the PAR (5.6 μM) and dark (2.2 μM) treatments. Similar increases in H_2O_2 concentration were found in Exp2a and Expl for UV radiation treatments (data not shown).

Discussion

Bacterial strains tested in this study were initially selected because their colonies proved to be dominant in the agar plate cultures obtained from Potter Cove seawater samples. The difficulty to identify these isolates on the bases of morphological and metabolic characteristics was not surprising because isolates from environmental samples frequently belong to taxa that are not included in the data base of standard test kits designed for clinical purposes (Busse et al. 1996). These systems were nevertheless useful for the characterization of bacterial strains when used in the context of chemo- and molecular taxonomic studies. Although the main objective of this paper was not

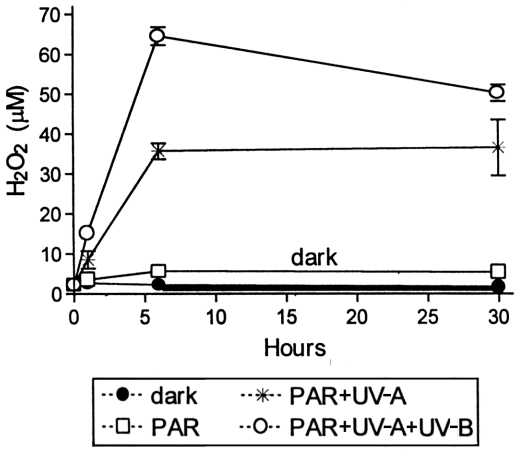

Fig. 6. Changes in hydrogen peroxide concentration during solar radiation exposure and the subsequent 24 h long dark period during Exp2b performed on 22 February 1999. Data obtained from dark (filled circles), PAR (open quadrants), PAR+UV-A (stars) and PAR+UV-A+UV-B (open circles) treatments are shown. Error bars indicate standard deviation of triplicates.

to make an exhaustive taxonomic study of the isolated strains, data obtained from partial sequencing of 16S rDNA showed some results that should be highlighted. The presence of an *Arthrobacter* strain in marine waters is not frequent. Members of this genus are commonly isolated from soils but some strains have been obtained from aquatic marine environments (Keddie et al. 1986). We cannot dismiss a terrestrial origin for the *Arthrobacter* UVvi strain, which could be associated with the sediments carried by the seasonal freshwater streams that are abundant in the area during the spring–summer period (Varela 1998). However, the salt dependency for growth suggests a marine origin for this strain. Loveland-Curtze et al. (1999) studied several psychrophilic strains of *Arthrobacter* and the only Antarctic isolate analysed by these authors (LV7, a yellow pigmented strain from a lake near the Miers and Adams glaciers), proved (like UVvi) to be phylogenetically related to *A. agilis* and presented a salt requirement for growth. In relation to the UVps strain, neither the physiological and morphological studies nor the partial sequencing of the 5' end 16S rDNA allowed us to assign it to any particular genus although their sequence is related to the members of the FCB group. This group is one of the most representative among the sea ice associated and coastal water Antarctic bacteria (Gosink & Staley 1995). Moreover, *Psychroserpens burtonensis* (the closest related strain based on the GenBank data base) is a taxa from Antarctic water samples that was only recently described (Bowman et al. 1997). This fact would explain the difficulty to identify the strain by the standard techniques and highlights the microbial biodiversity of the sea ice and Antarctic marine waters, which are still scarcely known (Staley & Gosink 1999).

Results presented here showed that the studied marine Antarctic bacteria are sensitive to the incident UV radiation, showing a major loss of viability under short times of exposure. The harmful effect of UV radiation on these marine bacteria is in accordance with others obtained from different non-Antarctic environments for both isolated strains (Arrage et al. 1993; Fernández & Pizarro 1996) and natural marine microbial communities (Müller-Niklas et al. 1995; Elasri & Miller 1999). But how sensitive are our strains? The level of sensitivity to UV radiation observed in FCB-related UVps and *Arthrobacter* UVvi was lower than that reported by Joux et al. (1999). These authors reported that when several marine strains were irradiated with 2.5 kJ m^{-2} of UV-B dose, all of them showed survival percentages lower that 10%. In contrast, while we observed different sensitivity in our strains to UV-B radiation, both of them showed higher survival percentages than those observed by Joux et al. at comparable UV-B doses. It is important to remark that these authors used only UV-B radiation from lamps for the experimental exposure, whereas in the assays presented here we used natural solar radiation. While the experimental differences prevent an adequate comparison between experiments, FCB-related UVps and *Arthrobacter* UVvi Antarctic strains seem to be less sensitive to UV-B irradiation than the non-Antarctic marine bacteria studied by Joux et al. (1999). In addition, different responses to PAR+UV-A+UV-B of the two strains were observed, FCB-related UVps being more sensitive than *Arthrobacter* UVvi. This difference was evident at UV-B doses lower than the 5 kJ m^{-2} range, at which *Arthrobacter* UVvi showed lower sensitivity than FCB-related UVps. For doses higher than 5 kJ m^{-2}, difference between strains was not observed. It is possible that at these high UV-B doses lethal effects mask the different sensitivities of the strains. The existence of this threshold effect is in agreement with our previous observations with these

strains (Hernández et al. 2002). Large interspecific differences in sensitivity to UV radiation and in the recovery capacity from previous UV stress among marine bacteria have been reported by other authors (Arrieta et al. 2000). Although the possible causes of these differences were not investigated in this assay, differences in sensitivity would not be related to the presence of effective UV-screening molecules, because this kind of compound has not been found in marine bacteria other than Cyanobacteria (Hader et al. 1998; Cockell & Knowland 1999).

Under diurnal stratification of coastal waters, micro-organisms confined to the surface layers are under high UV radiation levels. In our experiments, the strains were exposed to full irradiances, equivalent to those existing in the shallow surface layer (1 - 5 m). This condition, which resembles the situation occurring every day in tidal pools, is a common situation in surface waters of Potter Cove, where stratification was reported by Abele-Oeschger et al. (1997) based on the H_2O_2 values measured in surface waters during the day. Under these conditions, UV exposure could have an impact on near surface natural bacterial communities of Potter Cove. This effect of UV on surface bacterioplankton would not be an exclusive phenomenon of Potter Cove waters, because diurnal stratification is common in Antarctic areas as well as in many others oceanic regions (Doney et al. 1995).

Our results showed that UV-A contributes to a great extent to UV-induced death, in agreement with other authors (Helbling et al. 1995; Sommaruga et al. 1997). Although UV-B wavelengths are more harmful on a per photon basis, UV-A wavelengths comprise a much greater proportion of the UV radiation present in the solar spectrum and ambient sunlight UV-A could be eliciting a stronger biological response. Miller et al. (1999), working with Southern Ocean waters exposed to the same treatments designed by us, reported similar results: both UV-B and UV-A increase mortality in marine bacteria. However, unlike those results observed in our assays, they reported an increase in bacterial counts during the night in both UV-A and UV-B treatments. We also observed interspecific differences between isolates in relation to the recovery capacity in darkness after solar radiation exposure. In our case, the strain that showed the highest sensitivity to UV radiation (FCB-related UVps) showed no recovery capacity in darkness. In contrast, *Arthrobacter* UVvi strain, which seems to be more resistant to solar radiation, showed an increase in bacterial counts during the 24 h dark period after exposure to PAR + UV-A.

Although our assays were not designed to investigate the causes of this interspecific difference, such causes could be the consequence of differences in efficiency of one or several of the three different "dark" DNA repair mechanisms present in bacteria. In this sense, Huot et al. (2000) measured cyclobutane dimers and RecA (a key protein involved in the three DNA repair mechanisms) production pattern on microbial communities in the Gulf of Mexico and found that under natural daylight conditions, bacterioplankton exhibit a diurnal cycle with a maximum level of damage at 15:00 local time and a maximum RecA level at early evening. Similar results were reported by Miller et al. (1999) for Antarctic microbial communities. It is possible that *Arthrobacter* UVvi strain has more efficient dark DNA repair mechanisms than FCB-related UVps and that after the PAR + UV-A treatment, this strain is able to enhance the CFU ml^{-1} when the dark period is long enough. Evidently, damage suffered by FCB-related UVps under both PAR + UV-A + UV-B and PAR + UV-A treatments was enough to prevent any kind of recovery, at least under the conditions and time periods used in this study. It is important to remark that Winter et al. (2001), working with coastal North Sea bacterial communities, found that some members of the FCB group showed high sensitivity to UV radiation. This observation is in agreement with our observations working on FCB-related UVps in Potter Cove. The fact that, despite their UV sensitivity, these FCB members are persistent in both Noth Sea and Antarctic waters suggests that in nature these strains are not constantly exposed to the same radiation regime and UV dose suffered in the experiments performed. In Potter Cove, the strong winds and turbidity (produced by particulate material carried by the seasonal freshwater streams in the spring–summer period) could be generating vertical mixing and screen effect, respectively, that would markedly reduce the deleterious effect of solar radiation. However, as we mentioned above, the effect could be another when diurnal stratification occurs. In this case, UV radiation could be effective in the suppression of the UV-sensitive strains trapped in the euphotic layer. Also, any increase in the average UV levels (as that caused by the ozone hole) could be deeply influ-

encing species composition of the bacterial community. Due to the key role that bacteria play in providing the link to higher trophic levels in the food web and in mineralization of organic matter in aquatic ecosystems (Häder et al. 1998), the UV mediated changes in Antarctic aquatic bacterial communities would affect the aquatic ecosystem as a whole.

Finally, a high concentration of H_2O_2 in UV irradiated treatments compared with PAR and dark controls reflected the photoproducts formation from DOM (in these assays represented principally by peptone added to seawater). Such levels, which were higher than the highest values reported for Potter Cove surface waters under natural conditions (Abele et al. 1999), were in the range that we found previously as harmless for the studied strains (Hernández et al. 2002). In addition, after the dark period in the PAR+UV-A treatment of Exp2b, *Arthrobacter* UVvi was able to achieve 45 % of recovery despite H_2O_2 concentration at the end of the dark period was higher than 29 μM, confirming that H_2O_2 was not the cause of the bacterial mortality in these assays.

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References

Aas, P., Lyons, M. M., Pledger, R., Mitchell, D. L. & Jeffrey, W. H. 1996: Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of Mexico. *Aquat. Microb. Ecol. 11*, 229–238.

Abele, D., Ferreyra, G. A. & Schloss, I. 1999: H_2O_2 accumulation from photochemical production and atmospheric wet deposition in Antarctic coastal and off-shore waters of Potter Cove, King George Island, South Shetland Islands. *Antarct. Sci. 11*, 131–139.

Abele-Oeschger, D., Rottgers, R. & Tug, H. 1997: Dynamics of UV-driven hydrogen peroxide formation on an intertidal sandflat. *Limnol. Oceanogr. 42*, 1406–1415.

Arrage, A. A., Phelps, T. J., Benoit, R. E., & White, D. C. 1993: Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. *Appl. Environ. Microbiol. 59*, 3545–3550.

Arrieta, J. M., Weinbauer, M. G. & Herndl, G. J. 2000: Interspecific variability in sensitivity to UV radiation and subsequent recovery in selected isolates of marine bacteria. *Appl. Environ. Microbiol. 66*, 1468–1473.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. & Thingstad, F. 1983: The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser. 10*, 257–263.

Bader, H., Sturzenegger, V. & Hoigné, J. 1988: Photometric method for the determination of low concentrations of hydrogen peroxide by the peroxidase catalyzed oxidation of N,N-diethyl-p-phenylenediamine (DPD). *Water Res. 22*, 1109–1115.

Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. 1997: Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol. 63*, 3068–3078.

Busse, H. J., Denner, E. B. M. & Lubitz, W. 1996: Classification and identification of bacteria: current approaches to an old problem. Overview of methods used in bacterial systematics. *J. Biotechnol. 47*, 3–38.

Cockell, C. S. & Knowland J. 1999: Ultraviolet radiation screening compounds. *Biol. Rev. 74*, 311–345.

Davidson, A. T., Marchant, H. J. & de la Mere, W. K. 1996: Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed cultures. *Aquat. Microbial. Ecol. 10*, 299–305.

Delille, D., Bouvy, M. & Cahet, G. 1988: Short-term variation of bacterioplankton in Antarctic zone: Terre Adelie area. *Microb. Ecol. 15*, 293–309.

Doney, S. C., Najjar, R. G. & Stewar, S. 1995: Photochemistry, mixing and diurnal cycles in the upper ocean. *J. Mar. Res. 53*, 341–369.

Ducklow, H. W., Kirchman, D. L. & Quinby, H. L. 1992: Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the North Atlantic spring phytoplankton bloom, May, 1989. *Microb. Ecol. 24*, 125–144.

Elasri, M. O. & Miller, R. V. 1999: Study of the response of a biofilm bacterial community to UV radiation. *Appl. Environ. Microbiol. 65*, 2025–2031.

Fernández, R. O. & Pizarro, R. A. 1996: Lethal effect induced in *Pseudomonas aeruginosa* exposed to ultraviolet-A radiation. *Photochem. Photobiol. 64*, 334–339.

Gosink, J. & Staley, J. T. 1995: Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. *Appl. Environ. Microbiol. 61*, 3486–3489.

Häder, D. P., Kumar, H. D., Smith, R. C. & Worrest, R. C. 1998: Effects on aquatic ecosystems. *J. Phochem. Photobiol. B: Biol. 46*, 53–68.

Helbling, E. W., Marguet, E. R., Villafañe, V. E. & Holm-Hansen, O. 1995: Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. *Mar. Ecol. Prog. Ser. 166*, 293–298.

Hernández, E., Ferreyra, G. A. & Mac Cormack, W. P. 2002: Effect of solar radiation on two Antarctic marine bacterial strains. *Pol. Biol. 25*, 453–459.

Herndl, G. J., Arrieta, J. M., Kaiser, E., Obernosterer, I., Pausz, C. & Reitner, B. 2000: Role of ultraviolet radiation in aquatic systems: interaction between mixing processes, photochemistry and microbial activity. In C. R. Bell et al. (eds.): *Microbial biosystems: new frontiers. Proceedings of the 8th International Symposium on Microbial Ecology.* Pp. 209–219. Halifax: Atlantic Canada Society for Microbial Ecology.

Herndl, G. J., Muller-Niklas, G. & Frick, J. 1993: Major role of ultraviolet-B in controlling bacterioplankton growth in

surface layer of the ocean. *Nature 361*, 717–719.
Hugh, R. & Leifson, E. 1953: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J. Bacteriol. 66*, 24–26.
Huot, Y., Jeffrey, W. H., Davis, R. F. & Cullen, J. J. 2000: Damage to DNA in bacterioplankton: a model of damage by ultraviolet radiation and its repair as influenced by vertical mixing. *Photochem. Photobiol. 72*, 62–74.
Jeffrey, W. H., Pledger, R. J., Aas, P., Hager, S., Coffin, R. B., von Haven, R. & Mitchell, D. L. 1996: Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. *Mar. Ecol. Prog. Ser. 137*, 283–291.
Joux, F., Jeffrey, W. H., Lebaron, P. & Mitchell, D. L. 1999: Marine bacterial isolates display diverse responses to UVB radiation. *Appl. Environ. Microbiol. 65*, 3820–3827.
Kaiser, E. & Herndl, G. J. 1997: Rapid recovery of marine bacterioplankton activity after inhibition by UV radiation in coastal waters. *Appl. Environ. Microbiol. 63*, 4026–4031.
Karl, D. M. 1993: Microbial processes in the southern oceans. In E. I. Friedman (ed.): *Antarctic microbiology.* Pp. 1–63. New York: Wiley Press.
Keddie, R. M., Collins, M. D. & Jones, D. 1986: Genus *Arthrobacter* Conn and Dimmick 1947. In P. H. A. Sneath et al. (eds): *Bergey's manual of systematic bacteriology. Vol. 2*. Pp. 1004–1008. Baltimore: Williams and Wilkins.
Kim, D. S. & Watanabe, Y. 1994: Inhibition of growth and photosynthesis of freshwater phytoplankton by ultraviolet A (UVA) radiation and subsequent recovery from stress. *J. Plankton Res. 16*, 1645–1654.
Loveland-Curtze, J., Sheridan, P. P., Gutshall, K. R. & Brenchley, J. E. 1999: Biochemical and phylogenetic analyses of psychrophilic isolates belonging to the *Arthrobacter* subgroup and description of *Arthrobacter psychrolactophilus*, sp. nov. *Arch. Microbiol. 171*, 355–363.
Lubin, D., Frederick, J., Booth, C., Lucas, T. & Neuschuler, D. 1989: Measurements of enhanced springtime ultraviolet radiation at Palmer Station Antarctica. *Geophys. Res. Lett. 16*, 783–785.
Miller, R. V., Jeffrey, W., Mitchell, D. & Elasri, M. 1999: Bacterial responses to ultraviolet light. *ASM News 65*, 535–541.
Müller-Niklas, G., Heissenberger, A., Puškaric, S. & Herndl, G. J. 1995: Ultraviolet-B radiation and bacterial metabolism in coastal waters. *Aquat. Microb. Ecol. 9*, 11–116.
Saitou, N. & Nei, M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol. 4*, 406–425.
Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989: *Molecular cloning: a laboratory manual. 2nd edition*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Sommaruga, R., Oberleiter, A., Herndl, G. J. & Psenner, R. 1997: Inhibitory effect of solar radiation on thymidine and leucine incorporation by freshwater and marine bacterioplankton. *Appl. Environ. Microbiol. 63*, 4178–4184.
Staehelin, J., Harris, N. R. P., Appenzeller, C. & Ebenhard, J. 2001: Ozone trends: a review. *Rev. Geophys. 39*, 231–290.
Staley, J. T. & Gosink, J. J. 1999: Poles apart: biodiversity and biogeography of sea ice bacteria. *Ann. Rev. Microbiol. 53*, 189–215.
Varela, L. 1998: Hydrology of Matias and Potter creeks. *Ber. Polarforsch. 299*, 33–39.
Vincent, W. F. & Neale, P. J. 2000: Mechanisms of UV damage to aquatic organisms. In S. J. de Mora et al. (eds.): *The effects of UV radiation in the marine environment.* Pp 149–156. Cambridge: Cambridge University Press.
Winter, C., Moesender, M. M. & Herndl, G. J. 2001: Impact of UV radiation on bacterioplankton community composition. *Appl. Environ. Microbiol. 67*, 665–672.