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ORIGINAL ARTICLE

Diversity in UV sensitivity and recovery potential among bacterioneuston and bacterioplankton isolates

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Abstract

Aims: To assess the variability in UV-B (280–320 nm) sensitivity of selected bacterial isolates from the surface microlayer and underlying water of the Ria de Aveiro (Portugal) estuary and their ability to recover from previous UV-induced stress.

Methods and Results: Bacterial suspensions were exposed to UV-B radiation (3·3 W m^{-2}). Effects on culturability and activity were assessed from colony counts and ^{3}H-leucine incorporation rates, respectively. Among the tested isolates, wide variability in UV-B-induced inhibition of culturability (37·4–99·3%) and activity (36·0–98·0%) was observed. Incubation of UV-B-irradiated suspensions under reactivating regimes (UV-A, 3·65 W m^{-2}; photosynthetic reactive radiation, 40 W m^{-2}; dark) also revealed diversity in the extent of recovery from UV-B stress. Trends of enhanced resistance of culturability (up to 15·0%) and enhanced recovery in activity (up to 52·0%) were observed in bacterioneuston isolates.

Conclusions: Bacterioneuston isolates were less sensitive and recovered more rapidly from UV-B stress than bacterioplankton isolates, showing enhanced reduction in their metabolism during the irradiation period and decreased culturability during the recovery process compared to bacterioplankton.

Significance and Impact of the Study: UV exposure can affect the diversity and activity of microbial communities by selecting UV-resistant strains and alter their metabolic activity towards protective strategies.

Introduction

Since the discovery of the stratospheric ozone hole, concerns regarding the ecological consequences of UV radiation have extensively increased (Häder *et al.* 2007). In the face of a changing global environment, the assessment of the toxic nature of UV radiation, most notably in the UV-B wavelength (280–320 nm) that is expected to increase as a result of the interaction of changes in UV-B fluxes resulting from ozone depletion and other climate changes (UNEP 2010), has gained pertinence.

Bacteria play a key role in nutrient cycling in aquatic ecosystems (Azam and Malfatti 2007), but their small size, short generation times and the fact that their genome comprehends a large portion of the cell volume, might make them more susceptible to the effects of UV radiation than higher organisms (Garcia-Pichel 1994).

The main biological effect of UV-B results from UV-induced formation of covalent links between adjacent pyrimidine residues, usually known as cyclobutane pyrimidine dimers. These dimers cannot be replicated and are lethal to the cell, unless damage is repaired (Mitchell and Karentz 1993). In response to UV-induced damage, bacteria have evolved several repair mechanisms that can basically be divided in dark repair and photoreactivation, a light-dependent repair mechanism that uses the photolyase enzyme, activated by UV-A (320–400 nm) and photosynthetic reactive radiation (PAR, 400–700 nm) (Walker 1984).

Located at the air–water interface, the surface microlayer (SML) represents a stressful environment for microorganisms, where pollutants and heavy metals accumulate because of its lipophilic nature, and the intensity of solar UV radiation is at its highest (Maki 1993). Adding to

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their individual effects, pollutants and UV radiation can also act additively or synergistically on aquatic microbes (Pelletier *et al.* 2006), through the generation of photo-oxidative products that might impose an additional stress on bacterioneuston, i.e. bacteria inhabiting the SML. Reports of higher bacterial abundance and activities at the SML (Kuznetsova and Lee 2001) have suggested enhanced resistance of bacterioneuston to stress, most notably UV related. However, such a trend has not yet been demonstrated, and recent work has indicated similar UV resistance in bacterial isolates from the SML and underlying water (UW) (Agogué *et al.* 2005), although the authors only monitored the optic density of the cultures after the irradiation period. To our knowledge, information on the variability of the responses of bacterioneuston isolates in terms of culturability and activity, as well as their repair potential under different reactivating regimes following UV exposure, is virtually inexistent.

The aim of this work was the characterization and comparative analysis of the sensitivity of bacterial isolates from the SML and UW of the estuarine system Ria de Aveiro (Portugal) and the assessment of their repair potential under different light regimes after UV-B exposure. The variability in the extent of UV-induced inactivation and the influence of different light regimes on the recovery of selected marine bacterial isolates following UV-B stress were determined in laboratory experiments using artificial radiation.

Materials and methods

Sampling and isolation of marine bacterial strains

Bacterial strains were isolated from the SML and UW of the estuarine system Ria de Aveiro, located in the western coast of Portugal. Samples from the SML were collected using a glass plate sampler (Harvey and Burzell 1972). Samples from UWs were collected by submerging a polycarbonate bottle and opening it at a depth of 0·5 m. For the isolation of UV-resistant bacteria from the SML and UW, samples from both water layers were exposed to UV-B doses between 0 and 2200 J m^{-2} (Philips UV-B TL 100 W/01 lamp, maximum emission peak at 311 nm; intensity of 3·3 W m^{-2}), with agitation (50 rev min^{-1}) and at *c.* 20°C, as described by Fernández Zenoff *et al.* (2006a). Sample aliquots (100 μl) were removed at predetermined intervals and plated in marine agar 2216 plates (MA 2216; Difco, Detroit, MI, USA). After incubation in the dark at 20°C for 7–14 days, isolates were selected from the plates according to morphological differences and purified. Molecular typing of the isolates was performed by BOX-PCR, according to the procedure described by Rademaker *et al.* (1998), and isolates displaying distinct BOX profiles were identified by sequencing the 16S rRNA gene using the primer 27F and an ABI PRISM_BigDye_Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA). Sequences were compared with sequences available in the GenBank database by using the Basic Local Alignment Search Tool (BLAST) service to determine their approximate phylogenetic affiliations (Altschul *et al.* 1990). The sequences obtained were deposited in the GenBank database (see Table 1 for accession numbers).

Preparation of cell suspensions and irradiation conditions

Bacterial isolates growing in marine broth were harvested during the exponential phase by centrifugation (3200 ***g***, 15 min), and the pellet was washed three times with filtered-sterilized autoclaved sea water to remove all traces of the culture medium. Cells were resuspended in filtered-sterilized autoclaved sea water, and bacterial abundance was adjusted to 10^6 cells per ml, as determined by epifluorescent microscopy counts. Bacterial suspensions were transferred to sterile plates (Corning Science Products, Corning, NY, USA) without the lid and irradiated under the UV-B source used for the initial isolation for 20 min (corresponding to a final UV-B dose of 3·931 kJ m^{-2}). During irradiation, samples were kept at *c.* 20°C and incubated under slow shaking (50 rev min^{-1}). All experiments were conducted in the absence of ambient light to minimize photoreactivation. After irradiation, appropriate dilutions were plated on marine agar (MA 2216). Each experiment was conducted with triplicate replicates in four independent times.

Repair potential

To determine the repair properties of the bacteria, UV-B-irradiated cell suspensions were subjected to three different treatments: (i) photoreactivation with PAR, provided by white cool lamps (13·44 W m^{-2} s^{-1}; Philips TLD 58 W/84), (ii) UV-A provided by Philips TL 100W/10R lamps (wavelength range 350–400 nm; intensity 3·65 W m^{-2}) and (iii) darkness, by incubating the suspensions in the dark. Aliquots of samples were collected before and after incubation of the cells for 60 and 180 min under the different recovery regimes for culturable counts and bacterial activity assessment. Each experiment was conducted with triplicate replicates in four independent times.

Culturable counts

The UV-inactivation kinetics was followed by collecting triplicate 100-μl aliquots at predetermined intervals.

Table 1 Origin, phylogenetic affiliation, sequence similarity to the closest relative and NCBI accession number of the UV-resistant bacterial isolates used in this study

Origin	Strain	Bacterial group	Closest relative (accession no.)	% Sequence similarity	Accession no.*
SML	*Pseudomonas* sp. strain NT5I1.2B	γ-Proteobacteria	*Pseudomonas* sp. DSM 8628 (FM208263.1)	97	GU084169
	Paracoccus sp. strain NT25I3.1A	α-Proteobacteria	*Paracoccus* sp. JAM-AL07 (AB526330.1)	99	GQ365195
	Staphylococcus sp. strain NT25I2.1	Firmicutes	*Staphylococcus saprophyticus* ATCC 15305 (D83371.2)	99	GQ365197
	Micrococcus sp. strain NT25I3.2AA	Actinobacteria	*Micrococcus* sp. TA014 (EU308453.1)	98	GQ365196
	Sphingomonas sp. NT15I1.2B	α-Proteobacteria	*Sphingomonas* sp. PA225 (AM900788.1)	100	GU084171
UW	*Brevibacterium* sp. strain PT5I3.3L	Actinobacteria	*Brevibacterium casei* TSWCW1 (GQ284451.1)	99	GQ365205
	Bacillus sp. strain PT15I3.2CB	Firmicutes	*Bacillus cereus* 5YW6 (GU991861.1)	99	GQ365209
	Acinetobacter sp. strain PT5I1.2G	γ-Proteobacteria	*Acinetobacter* sp. BS8Y (EU545154.1)	97	GQ365202
	Psychrobacter sp. strain PT15I3.2CA	γ-Proteobacteria	*Psychrobacter piscidermidis* P4-4 (EU127295.1)	99	GQ365208

SML, surface microlayer; UW, underlying water.
*Accession numbers based on 16S rRNA partial gene sequence.

Aliquots were serially diluted in aged 0·2-μm-filtered autoclaved sea water and plated in Marine agar 2216 plates. Colonies were counted after 2–7 days of incubation in the dark at 20°C. The fraction of surviving cells was calculated by dividing the number of CFU in the treated sample by the number of CFU in the unirradiated sample at time zero. The dilution and plating procedures were carried out under low-luminosity conditions to avoid photoreactivation.

Bacterial activity

The bacterial activity of cell suspensions before irradiation, after irradiation and after reactivation was assessed from the rates of protein synthesis estimated by the incorporation of [^{3}H] leucine (Amersham; specific activity 63·0 Ci mmol^{-1}) into bacterial protein at 480 nmol l^{-1} final concentration in triplicate aliquots (1·5 ml) and one trichloracetic acid (TCA; Sigma, St Louis, MO, USA) fixed blank (2% final concentration). After 1 h of incubation in the dark, proteins were precipitated by the addition of 20% TCA and samples were centrifuged. TCA-washed pellets were resuspended in 1·5 ml of Universol liquid scintillation cocktail (ICN Biomedicals, USA). The radioactivity incorporated into bacterial cells was counted in a Packard Tri-Carb 2000 Liquid Scintillation Counter using the external standard ratio technique (Simon and Azam 1989).

Results

In this study, UV-resistant strains isolated after irradiation of samples from the SML and UW were used to assess the variability in UV-B sensitivity and recovery potential in bacterioneuston and bacterioplankton isolates. UV-B-induced inhibition of culturability ranged from 37·4% in *Micrococcus* sp. (NT25I3.2AA) (Fig. 1a) to 99·3% in *Staphylococcus* sp. (NT25I2.1) (Fig. 1e). The reduction imposed by UV-B exposure on bacterial activity ranged from 36·0% in *Staphylococcus* sp. (NT25I2.1) (Fig. 1e) to 98·0% in *Bacillus* sp. (PT15I3.2CB) (Fig. 2b). On average, UV-B-induced reduction in bacterial culturability was up to 15% higher in bacterioplankton isolates, while the reduction in the activity was up to 25% higher in bacterioneuston isolates (ANOVA, $P < 0{\cdot}05$) (Figs 1 and 2).

Recovery of culturability under the UV-A (up to 36·4%) and PAR (up to 27·6%) regimes was observed for eight of nine of the isolates tested (Figs 1 and 2). Recovery in bacterial culturability (up to 29·1%) was also observed under the dark regime for six of the nine isolates tested (Figs 1 and 2). The bacterioneuston isolate *Micrococcus* sp. strain NT25I3.2AA (Fig. 1a) showed the highest recovery efficiency (up to 36·4%) under all regimes tested. In general, significant differences between the patterns of recovery under the different light regimes were not detected. However, when considering the SML and UW in separate, differences emerged. For SML isolates, the most favourable light regime for the recovery of bacterial culturability was UV-A (average recovery of 11·4%), while PAR induced the lowest recovery rate (6·4%). For bacterioplankton isolates, PAR led to the highest recovery rates (9·5%), while the lowest recovery was observed under UV-A irradiation (4·6%).

Bacterial activity also recovered, at variable extensions under the different light regimes (Figs 1 and 2). In general, UV-A radiation was the most favourable light regime in the recovery of bacterial activity (up to 78·4%), while significant differences between recovery under PAR (up to 14·6%) and in the dark (up to 17·2%) were not found

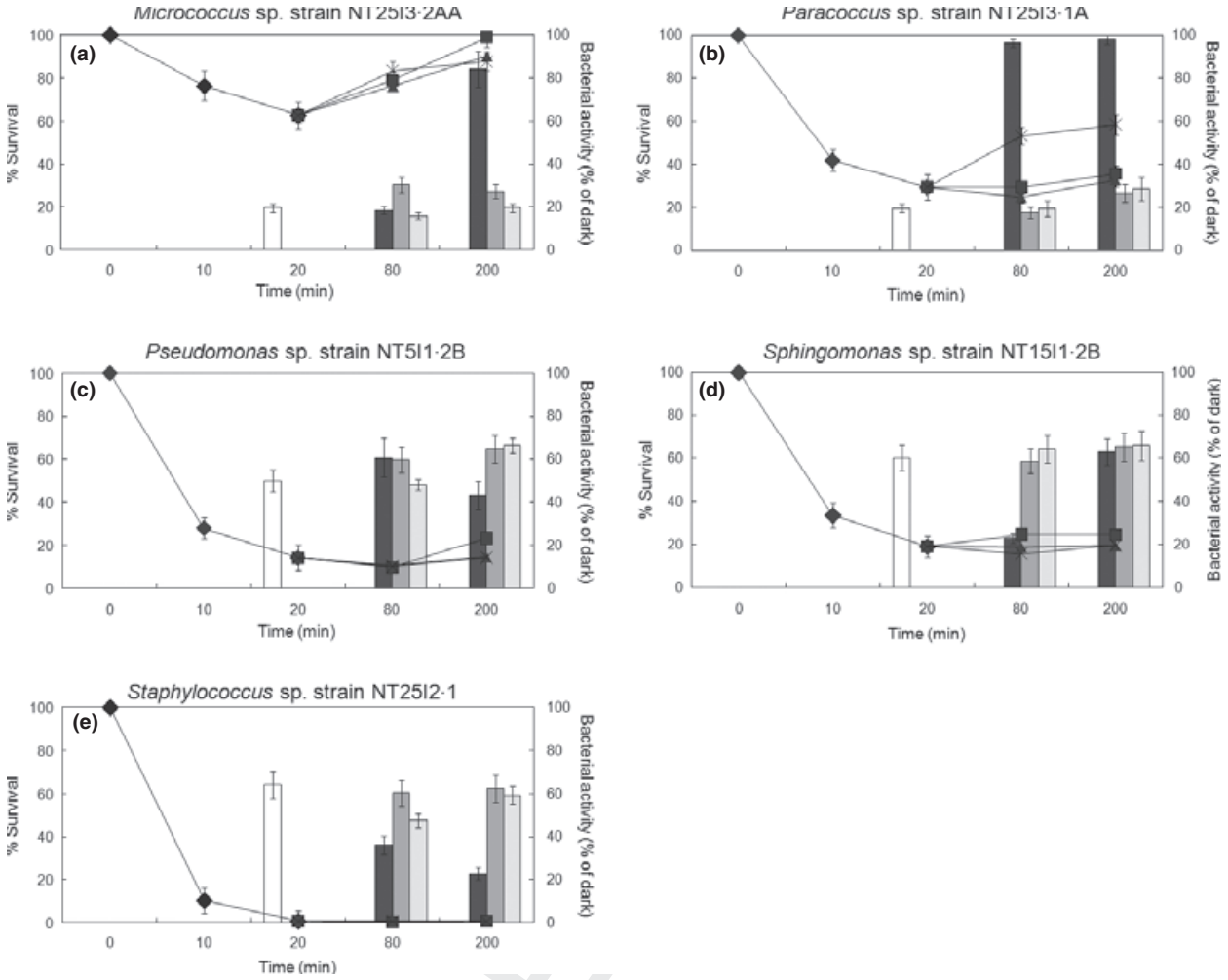

Figure 1 Effects of UV-B radiation and reactivation under different light regimes on the culturability and activity of UV-resistant bacterioneuston isolates. Cells were exposed to UV-B radiation for 20 min and allowed to recover under different reactivation regimes (UV-A, PAR, dark) for 180 min. Aliquots were collected after 60 min and 180 min of recovery for cultivation and activity measurements. Curves and bars in the figures correspond to the variation in culturability and activity rates, respectively. Error bars represent standard deviations of triplicate replicates of four independent experiments. Absence of error bars indicates standard deviations are too small to see on the scale used. Results are expressed as % of the unirradiated sample at time zero. (□) UV-B; (■) UV-A; (▩) PAR; (□) dark; (–◆–) UV-B; (–■–) UV-A; (–▲–) PAR and (–×–) dark. PAR, photosynthetic reactive radiation.

($P > 0{\cdot}05$, one-way ANOVA). Bacterioneuston isolates *Paracoccus* sp. strain NT25I3.1A (78·4%) (Fig. 1b) and *Pseudomonas* sp. strain NT5I1.2B (14·6%) (Fig. 1c) showed the highest recovery rates in bacterial activity under the UV-A and PAR regime, while the planktonic isolate *Bacillus* sp. strain PT15I3.2CB (17·2%) (Fig. 2b) recovered the most under the dark regime. When considering the compartment from which the bacterial isolates were retrieved, the patterns observed for bacterial culturability were maintained; though for bacterioplankton isolates, significant differences were observed in the extent of the recovery in the dark and under UV-A radiation (twice as high than under the dark regime).

Discussion

Several studies on UV sensitivity and recovery potential in bacteria from diverse aquatic environments have been conducted (Joux *et al.* 1999; Arrieta *et al.* 2000; Agogué *et al.* 2005). However, to our knowledge, no information exists on the diversity of the UV responses of bacterioneuston isolates in terms of culturability, activity and recovery from previous UV-induced stress. Therefore, in this study, the variability in the UV sensitivity and recovery potential of selected UV-resistant bacterioneuston and bacterioplankton isolates was assessed under standardized experimental conditions using a number of isolates simi-

LOW RESOLUTION FIG

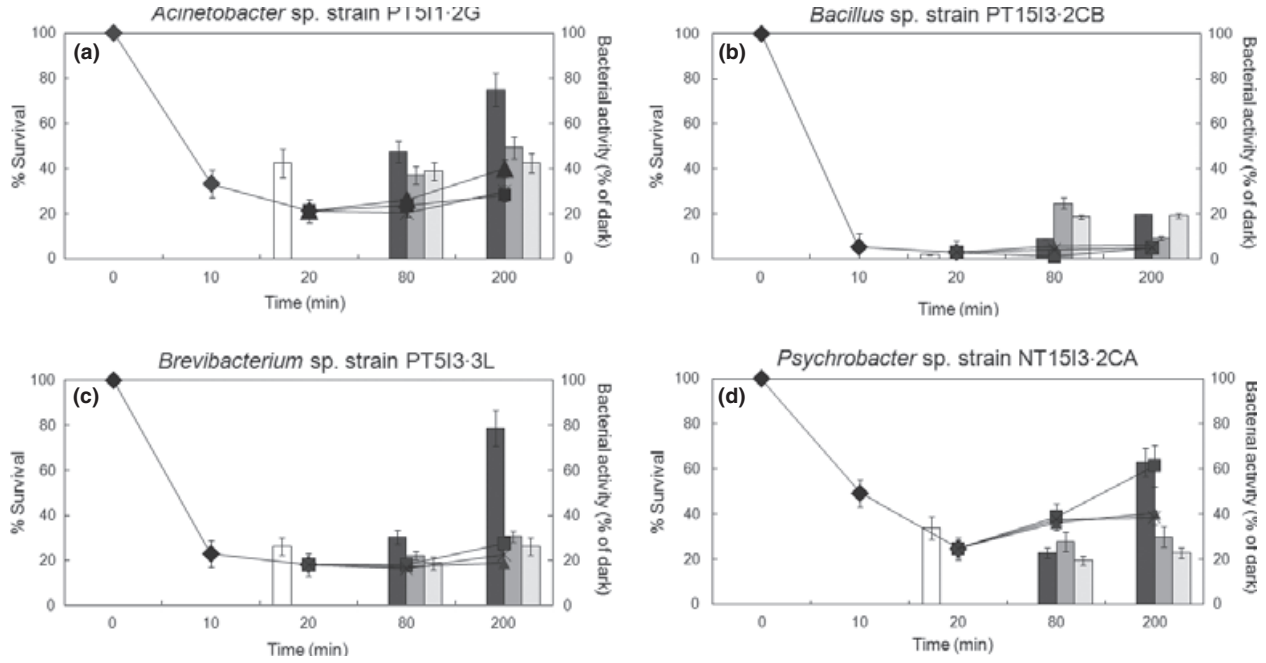

Figure 2 Effects of UV-B radiation and reactivation under different light regimes on the culturability and activity of UV-resistant bacterioplankton isolates. Cells were exposed to UV-B radiation for 20 min and allowed to recover under different reactivation regimes (UV-A, PAR, dark) for 180 min. Aliquots were collected after 60 min and 180 min of recovery for cultivation and activity measurements. Curves and bars in the figures correspond to the variation in culturability and activity rates, respectively. Error bars represent standard deviations of triplicate replicates of four independent experiments. Absence of error bars indicates standard deviations are too small to see on the scale used. Results are expressed as % of the unirradiated sample at time zero. (□) UV-B; (■) UV-A; (▩) PAR; (▢) dark; (–◆–) UV-B; (–■–) UV-A; (–▲–) PAR and (–×–) dark. PAR, photosynthetic reactive radiation.

lar to previous studies (Joux *et al.* 1999; Arrieta *et al.* 2000; Fernández Zenoff *et al.* 2006b).

Exposure to UV-B radiation revealed considerable variability in sensitivity to UV stress among the tested isolates, in agreement with previous reports (e.g. Arrieta *et al.* 2000; Ordoñez *et al.* 2009). *Micrococcus* sp. (NT25I3.2AA) showed the smallest reduction in culturability upon UV-B exposure. High UV resistance of several *Micrococcus* strains isolated from other UV-exposed environments is well documented in the literature (e.g. Fernández Zenoff *et al.* 2006a,b; Ordoñez *et al.* 2009). The characteristically high G+C content of Actinobacteria has been proposed to confer a protective effect against UV radiation, by protecting DNA against damage by thymidine dimerization (Singer and Ames 1970). However, recent work (Matallana-Surget *et al.* 2008) has suggested that micro-organisms with high GC content could, in fact, be more prone to UV-induced mutations, because cytosine-containing photoproducts are highly mutagenic. The determinants of UV resistance in *Micrococcus* sp. remain, therefore, unknown.

Considerable differences were also observed in the extent of UV-induced inhibition of bacterial activity. The observation that *Staphylococcus* sp. (NT25I2.1) was the strain most inhibited in terms of culturability but less inhibited in terms of activity is intriguing, as one would expect the two biological parameters to be correlated. A similar trend was also observed in *Micrococcus* sp. (NT25I3.2AA) that was least affected in terms of culturability but was the second most affected strain in terms of activity (80·4% inhibition). A possible explanation would be the induction of a metabolic shift from growth (protein synthesis) to survival upon UV-B exposure, recently proposed to occur in *Bacillus cereus* in response to acid stress (Mols *et al.* 2010). Further studies are needed to reveal whether such a response also occurs upon UV-B stress.

The most important biological effects of UV-B radiation are probably a consequence of the stalling of replication fork complexes by UV-B-induced DNA lesions that block replisome movement or synthesis by the polymerase subunits. Therefore, the ability of bacteria to survive UV-B radiation is closely related to their ability to either bypass or correct such damaged DNA segments (Friedberg 1985).

Following UV-B exposure, the recovery potential of the bacterial isolates was assessed under different light regimes. UV-A and PAR were the most effective regimes

in the recovery of culturability, but recovery under the dark regime was also observed for most isolates. Such observation demonstrates the importance of photoreactivation, and particularly the enzyme photolyase, in the recovery from UV-B stress, in accordance with previous studies (e.g. Kaiser and Herndl 1997). The importance of photoreactivation for bacterial communities might be related to its extreme efficiency, splitting approximately one dimer for every blue–light photon absorbed. Furthermore, unlike dark repair, photoreactivation does not require energy mobilization and may be particularly important in nutrient-limited aquatic microbial populations (Joux *et al.* 1999). Recovery in terms of bacterial activity was also observed under the different light regimes, demonstrating that UV-induced impairment of biological functions was not irreversible being, in general, UV-A the most effective light regime for activity recovery.

Although the number of isolates used in this study is too small to attempt the establishment of a correlation between the origin of the isolates and their UV sensitivity, some trends were in fact identified. For example, UV-induced reductions in bacterial culturability were up to 15% lower in bacterioneuston, suggesting the presence of bacteria displaying enhanced resistance to UV radiation at the SML. The average recovery in terms of culturability was generally higher in bacterioplankton isolates (up to 33·5%) than in bacterioneuston for all light regimes. On the other hand, in terms of activity, the recovery was higher in bacterioneuston (up to 52·0%) under all the reactivating conditions tested. The observation of enhanced recovery in activity accompanied by reduced recovery in culturability in bacterioneuston isolates could indicate the engagement in a viable but nonculturable condition as a stress response and/or defence mechanism that allows for the rapid re-establishment of bacterial activity when UV exposure is terminated. This hypothesis is supported by the observation that the UV-induced decrease in total cell numbers, determined by epifluorescence microscopy counts of acridine orange-stained preparations, was much lower than the one of CFU numbers (data not shown). Such a strategy has been reported, for example, in species of the genus *Vibrio* sp. upon exposure to thermal, saline and acidic stress (Wong and Wang 2004).

While addressing the resistance of bacterioneuston and bacterioplankton strains to solar UV radiation, by directly isolating culturable bacteria from the SML and UWs and monitoring the optic density of the irradiated cells, Agogué *et al.* (2005) concluded that UV resistance was similarly distributed in bacterioneuston and bacterioplankton. Alternatively, in this study, samples from the SML and UW were exposed to UV-B radiation and resistant bacteria were isolated (Fernández Zenoff *et al.* (2006a)). This approach may offer a more realistic perspective on bacterial UV resistance in a context of increased UV-B levels (UNEP, 2010), because the strains retrieved represent the dominant members of the culturable fraction of bacterioneuston and bacterioplankton challenged with elevated UV-B radiation, and the metabolic strategies they adopt to cope with this stress may modulate the overall functioning of the communities exposed to heighted UV-B doses.

The differences in the UV sensitivity of bacteria inhabiting the SML and UW that seem to emerge by the selective elimination of sensitive phenotypes and the enrichment in resistant strains can indicate the presence of UV-resistant members in the bacterioneuston population.

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