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Response to UVB radiation and oxidative stress of marine bacteria isolated from South Pacific Ocean and Mediterranean Sea

S. Matallana-Surget [a,b,*], C. Villette [a,b,c], L. Intertaglia [d,e], F. Joux [a,b], M. Bourrain [a,b,c], P. Lebaron [a,b,d,e]

[a] UPMC Univ Paris 06, UMR7621, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, F-66650 Banyuls/mer, France
[b] CNRS, UMR7621, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, F-66650 Banyuls/mer, France
[c] Centre de Recherche Dermatologique Pierre Fabre, BP 13562, 31 035 Toulouse, France
[d] UPMC Univ Paris 06, UMS 2348, Observatoire Océanologique, F-66650 Banyuls/mer, France
[e] CNRS, UMS 2348, Observatoire Océanologique, F-66650 Banyuls/mer, France

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ABSTRACT

Marine bacterial strains isolated from South Pacific and Mediterranean Sea were studied for their resistance to UVB radiation, their repair capacity under photoreactivating light, as well as their oxidative stress response using concentrated hydrogen peroxide (H_2O_2), as an oxidizer. A total of 30 marine bacteria were isolated from the hyper-oligotrophic waters of the South Pacific Gyre to the eutrophic waters of the Chilean coast during the BIOSOPE cruise (2004), and 10 strains from surface Mediterranean coastal waters. One third of bacteria presented a high resistance to UVB and almost all isolates presented an efficient post-irradiation recovery. Only few strains showed cell survival to high concentration of H_2O_2. No correlation between the sampling sites and the bacterial UVB resistance was observed. Two marine bacteria, *Erythrobacter flavus* and *Ruegeria mobilis*, were of particular interest, presenting a good response to the three parameters (UVB and H_2O_2 resistance/efficient repair). Unexpectedly, two resistant strains were again identified as *Ruegeria* species underlining that this geographically widespread genus, resist to UVB regardless the environment from which the isolates originate.

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1. Introduction

Marine bacteria inhabiting the ocean surface must face up to various stresses such as the damaging ultraviolet radiation (UVR and 280–400 nm). Different studies have demonstrated that both UVB (280–315 nm) and UVA (315–400 nm) fractions impair organisms in aquatic ecosystems, thus affecting biogeochemical cycles [1]. Photobiological studies of marine bacteria can be of great help to understand how these microorganisms can survive in surface waters exposed to high UVR fluxes [2,3], since they are especially vulnerable due to their small size, limiting effective cellular protections [4]. It is well known that UVB radiation causes both indirect and direct damage to DNA because of the strong absorption of this macromolecule at wavelengths below 315 nm. On one hand, the two most common photoproducts induced by UVB are namely the cyclobutane pyrimidine dimer (CPDs) and the pyrmidine (6–4) pyrimidone photoproducts (6–4PPs). Hopefully, bacteria also efficiently repair those photoproducts through two main pathways (i) the light-dependent photoenzymatic repair (PER) and (ii) the light-independent repair processes, including the nucleotide excision repair (NER). The recovery from previous UVB stress is higher under visible light than in the dark [5,6]. On the other hand, the UVB fraction, such as the UVA radiation range, may also cause indirect damage to proteins and lipids through the formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). H_2O_2 has been implicated in the oxidative stress-induced mortality of bacteria originated from diverse aquatic environments [7–10] due to damage induced to nucleic acid, proteins, and lipids [11].

The level of damaging effects induced by UVR in marine ecosystems is largely dependent of the UVR penetration that is highly variable according to the water transparency [12]. Oligotrophic oceans are characterized by transparent waters with a deeper UVR penetration compare to coastal waters [13]. In this way, microorganisms inhabiting oligotrophic waters should be severely affected [14]. Climate change is expected to reinforce the thermal stratification of oceans resulting in both the increased exposure of organisms to solar radiation in surface waters and an expansion of oligotrophication [1,15]. It was previously reported that UVR has little effect on the composition of marine bacterioplankton communities harvested from the surface mixed layer of the coastal North Sea [16]. Nevertheless, bacterial sensitivity to UVR is not uniform from one group to another. Recently, Ruiz-González et al. [17] observed that SAR11 clade was more sensitive to UVR

* Corresponding author at: UPMC Univ Paris 06, CNRS, UMR7621, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, F-66650 Banyuls/mer, France. Tel.: +33 (0)4 68 88 73 18.

E-mail address: sabine.matallana@obs-banyus.fr (S. Matallana-Surget).

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compare to *Gammaproteobacteria* and *Bacteroidetes*. Regarding the culturable fraction of bacteria, previous studies raised the question whether marine bacteria inhabiting the aquatic surface microlayer display higher resistance to UVR than underlying communities. While UVR resistance was found to be similar for bacterial strains isolated from both the sea surface microlayer and underlying waters in the north-western Mediterranean Sea [18], an opposite trend was observed in the estuary of Ria de Aveiro (Portugal), where the level of UVB resistance was shown to be significantly different in those two layers, with a higher resistance for the species inhabiting the surface waters [19]. Those differences might be the result of different environmental conditions.

By studying several sampling points from the large transect of ~8000 km investigated in the South East Pacific and in the Mediterranean Sea, we first aimed to provide a comparison of the response of selected bacteria, isolated from different environments ranging from ultra-oligotrophic to eutrophic waters, to three parameters: UVB/H_2O_2 resistance and recovery capacity. From this comparison, we were seeking for the identification of new culturable strains resistant to UVB, oxidative stress, with efficient repair ability and for relationships between the original environment of sampling (location and depth) and the responses of the bacterial strains.

2. Materials and methods

2.1. Isolation and cultivation of marine bacteria

The bacterial strains used in this study were provided by the MOLA collection at the Observatoire Océanologique in Banyuls-sur-mer (http://collection.obs-banyuls.fr). The selected strains were isolated from the South East Pacific during the BIOSOPE (BIogeochemistry and Optics SOuth Pacific Experiment) cruise during austral summer 2004 and from north-western Mediterranean Sea coastal water in September 2010. The BIOSOPE cruise consisted of 8000 km of transect according to a trophic gradient starting from the mesotrophic area of Marquesas archipelago, the extremely oligotrophic area associated with the central part of the South Pacific Gyre, and ended off with the upwelling region of coastal waters of Chile (Fig. 1) [20]. In the hyper-oligotrophic waters of the South Pacific Gyre, diffuse attenuation coefficients for downward irradiance (Kd) at 305 nm were 0.083 m^{-1} and 0.756 m^{-1} in the upwelling region [21]. The former UVB penetration is the highest ever reported for oceanic waters. Furthermore, a recent study reported that UVB and UVA doses measured at the sea surface in the South East Pacific are respectively as high as 0.3 and 11 kJ m^{-2} nm^{-1} for the whole day in summer [14]. Bacterial strains were isolated from samples collected between 5 and 50 m depth from two oligotrophic [GYR (114.01W; 26.06S); STB12 (104.31W; 28.54S)] and two eutrophic environments [STA21 (75.84W; 33.58S); UPX (73.39W; 33.98S)] [20] by spreading 100-μl of seawater on Marine Agar (MA, BD Difco™) plates incubated at ambient temperature. Samples from NW Mediterranean Sea were collected in the Banyuls/mer Bay (France, 42°29″N ; 03°08″E) at noon on a sunny day in September 2010 close to the surface (0.5 m). In summer, this area presents a high daily dose of UVB (up to 44 kJ m^{-2}) and a relatively high transparent waters (Kd_{305nm} = 0.5 m^{-1}) [22]. Immediately after the sampling, samples were disposed in sterile petri dishes without cover and exposed to artificial UVB radiation during 3 h (see below). For isolation of the UVB resistant bacterial strains, 100-μl of irradiated seawater were spread on MA plates. After incubation in the dark at 25 °C for 1–2 days, isolates were selected from the plates according to differences in color and shape. A total of 10 isolates were then picked and purified, and were referred to as BAN (states for BANYULS) with an associated number ranging from 1 to 10. Those 10 candidates resistant to UVB radiation were subsequently submitted to the same screening workflow than the one used for the BIOSOPE collection.

For cultivation, bacteria were grown in batch culture in marine broth (MB; BD Difco™) medium with continuous stirring at 25 °C. The bacterial growth was monitored by measuring the optical density (OD) at 620 nm. Growth in minimal medium of artificial seawater (ASW) containing 3 mM glucose (ASW-G) [23] was used instead of MB for further experiments on four bacterial strains that showed a high resistance to UVB and good recovery in MB (MOLA367, MOLA368, BAN3, BAN10).

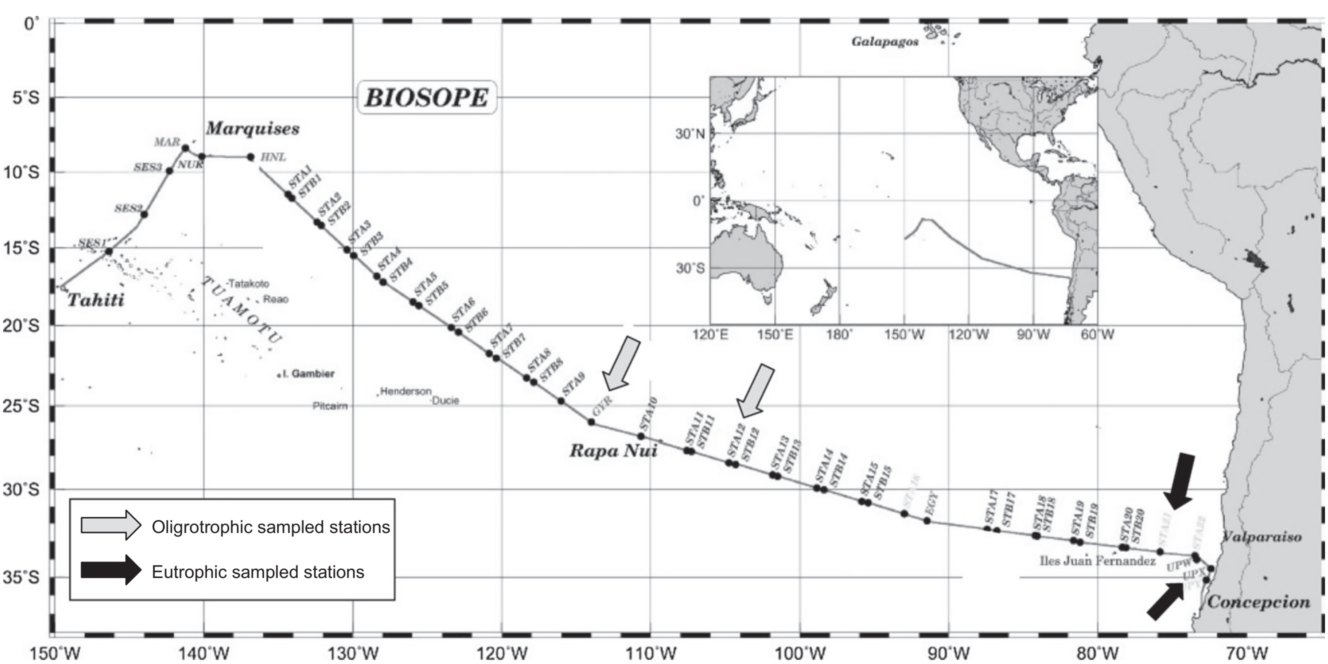

Fig. 1. Sampled stations from the BIOSOPE cruise. The arrows indicate the oligotrophic (GYR and STB12) and eutrophic (STA21 and UPX) environments used in our study. Figure modified from Claustre et al. 2008 [20].

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