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Effects of UV radiation on the taxonomic composition of natural bacterioplankton communities from Bahía Engaño (Patagonia, Argentina)

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ABSTRACT

In order to gain insights into the effects of solar ultraviolet radiation (UVR, 280–400 nm) on the composition of marine bacterioplankton communities from South Atlantic waters – Bahía Engaño (Patagonia, Argentina), we performed microcosms experiments during the Austral summer of 2010. Water samples were exposed to three solar radiation treatments in 25 L microcosms during 8 days: PAR + UV-A + UV-B (280–700 nm; **PAB** treatment), PAR + UV-A (320–700 nm; **PA** treatment), and PAR only (400–700 nm; **P** treatment). The taxonomic composition of the bacterial communities, at the beginning and at the end of the experiment, were studied by the analyses of 16S rDNA gene libraries. Multivariate and phylogenetic analyses demonstrated substantial differences in the community composition so that the samples exposed to PAR and PAR + UV-A presented more similar taxa assemblages among them than compared to the PAR + UV-A + UV-B exposed one. Our results indicate that overall, exposure to different radiation treatments can shape the taxonomic composition of marine bacterial populations, grown in microcosms, from this Patagonian area.

1. Introduction

For a long time, microorganisms were considered to be a negligible component of ecosystems. However, during the past century, it has been recognized that these organisms are important component of the biota, playing a key role in aquatic ecosystems [1,2]. In particular, it has been shown that marine bacteria are extremely abundant (10^6 cells ml^{-1}) and that they account for most of the biomass and metabolism in the oceans [3,4]. Prokaryotic cells are involved in biogeochemical cycles, affecting the availability of H, C, N, O, S and P for biological production [5], with its consequent impact on the Earth's climate. On the other hand, bacteria are responsible for the so called 'microbial loop', the process by which dissolved organic matter (DOM) is recycled into the food webs [6,7].

In aquatic environments, ultraviolet radiation (UVR, 280–400 nm), whether or not enhanced by ozone depletion, has in general negative effects on different cellular targets such as the DNA molecule, the photosynthetic apparatus or the membrane lipids, altering different cell processes like growth, photosynthesis, or even leading to cells' death [8]. In addition, UVR can induce changes in the cell size distribution, the taxonomic composition of particular aquatic groups such as diatoms [9], as well as changes in the morphology of microorganisms [10]. Several studies focused on the role of UVR on marine bacteria, have provided evidence that the most energetic waveband – UV-B (280–315 nm) – may induce more detrimental effects on bacterial DNA than on other planktonic cells [11,12]. Besides these direct effects of solar radiation, bacteria can also be affected by indirect mechanisms. For example, changes in phytoplankton composition and biomass, induced by UVR, are known to influence bacterial community composition (BCC) [13]. Likewise, predation by heterotrophic nanoflagellates [14] or bacteriophages [15] is also affected by UVR, therefore UVR-induced changes of these mortality factors could also affect BCC.

It is well established that UVR has significant negative effects on productivity, activity and abundance of bacterial cells [16–18]; however, there is no consensus regarding the effects of UVR on BCC. In humic waters, for example, it has been demonstrated that there is a relation between BCC and the depth attenuation of solar radiation [19]. Studies performed with marine bacterial isolates

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have shown that there are interspecific differences of particular bacterial groups regarding their sensitivity to UVR, together with differences in the mechanisms of recovery from UVR-induced stress [20,21]. Similar results were observed in experiments carried out with whole natural bacterioplankton communities from marine environments, in which it was observed that different groups appeared to have contrasting sensitivities to UVR [22–24]. Moreover, short-term experiments carried out by Santos et al. [25] revealed that UVR had clear effects on the abundance, structure, and function of estuarine bacterial communities. The studies mentioned above suggest that UVR affects the microbial community structure in marine waters. Conversely, experiments performed with diluted coastal marine bacterioplankton from the North Sea, grown in predator-free microcosms, suggested a moderate effect of UVR on BCC [26], whereas experiments carried out with Antarctic bacterial assemblages demonstrated that UVR induced only subtle effects on BCC [27].

Studies focusing on bacterial diversity and community composition of marine Patagonian environments are rather scarce [28]. Patagonian aquatic environments has unique characteristics (such as high heliofany and high solar radiation levels during spring-summer, proximity to the Antarctic Polar vortex, and high productivity in waters over the Continental Shelf) which constitute interesting scenarios for assessing the effects of UVR on aquatic organisms [9,29,30]. In order to gain insights into the potential effects of UVR on the composition of bacterial communities from Patagonia, we exposed whole planktonic communities collected from Bahía Engaño (near the Chubut River estuary) to different solar radiation treatments during 8 days. The effects of these treatments on BCC were assessed by sequence analysis of rDNA gene libraries.

2. Materials and methods

2.1. Site description and experimental setup

The study was carried out during the austral summer (January 20–28, 2010) with whole natural plankton samples collected from Bahía Engaño, near the Chubut River estuary (Chubut Province, Argentina). The Chubut River, the most important of the Chubut Province, flows into the Atlantic Ocean forming a coastal-plain meso-tidal estuary [31]. The area is characterized by a wide range of physical, chemical, and biological variables, as well as by the nutrients input carried by the river, which is associated with a relatively high phytoplankton biomass [9]. The sample used for experimentation (~200 L of seawater) was collected from surface waters during high tide (seawater condition) from the station named EGI, located at Northern coastal Patagonia (43°21′35″S; 65°00′41″W). The sample was collected in acid-cleaned opaque carboy-tanks and was immediately taken to the laboratory for experimentation. An initial filtration of the entire sample was carried out through a 100 μm pore size mesh (Nitex®). An aliquot was kept for direct analyses of the BCC at the initial time (T_0) and the rest of the sample was used to set-up six microcosms (25 L of capacity) which consisted of UVR-transparent bags. These microcosms were placed in a tank (3 m diameter, 1 m depth) with running water for temperature control and exposed to solar radiation during 8 days. Due to the timing of the sampling (summer), that is considered a post-bloom condition in our study area [32], macronutrients were added to each bag at the beginning of the experiment to avoid nutrient constraints – nitrate (8.82×10^{-4} M), phosphate (3.62×10^{-5} M) and silicate (1.06×10^{-4} M); all concentrations were as in the *f*/2 medium [33]. Three different radiation conditions (duplicate for each radiation treatment) were implemented: (1) **PAB** treatment, samples receiving full solar radiation (PAR + UV-A + UV-B, 280–700 nm), uncovered bags; (2) **PA** treatment; samples receiving PAR + UV-A (320–700 nm); bags covered with Folex 320 filter (50% transmittance at 320 nm); and (3) **P** treatment, samples receiving only PAR (400–700 nm); bags covered with Ultraphan 395 filter (50% transmittance at 395 nm). No significant differences were determined among them with respect to the incident PAR (<4% transmission) reaching the samples.

2.2. Measurement and analyses

The following measurements and analyses were performed:

2.2.1. Solar radiation

Incident solar radiation over the study area was monitored continuously using a broad-band European Light Dosimeter Network (ELDONET) radiometer (Real Time Computers) that is permanently installed at the roof of the Estación de Fotobiología Playa Unión (EFPU). The instrument measures UV-B (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm) with a frequency of one reading per second and stores the minute-averaged values for each channel.

2.2.2. DNA extraction

Samples for DNA analyses were collected at the beginning (T_0) and from each treated microcosm at the end of the experiment. Each aliquot (500–2000 mL) was filtered through different pore sized (20; 10; 5 and 0.22-μm) polycarbonate membrane filters (47 mm, MSI Westboro) to separate the different cell size fractions.

Whole community genomic DNA was extracted from each filter as described elsewhere [8,34]. Briefly, the filters were incubated at 60 °C for 30 min with 720 μL of pre-heated CTAB buffer (2% [w/v] CTAB Sigma, 1.4 M NaCl, 0.2% [v/v] β-mercaptoethanol, 20 mM EDTA, 100 mM Tris–HCl pH 8.0). A volume of 720 μL chloroform/isoamyl alcohol (24:1) was added and after centrifugation (20000 g, 15 min), the aqueous phase was transferred to a clean tube and one volume of cold isopropanol was added to precipitate the DNA (1 h at 4 °C). After centrifugation at 20000 g for 30 min at 4 °C, the supernatant was removed and the pellet was washed with 70% of ice-cold ethanol. The DNA pellet was dried and resuspended in ultrapure DNAase free water (Invitrogen). An aliquot of the DNA recovered from each filter was analyzed by gel electrophoresis on a 0.8% agarose gel in Tris–Acetate–EDTA buffer (TAE, Invitrogen) (1 h, 80 V), stained with ethidium bromide and visualized under UV light. The amount of DNA recovered from each filter was quantified by densitometric analysis using the ImageJ software [35], against a standard curve prepared by quantitating a molecular weight standard (High DNA Mass Ladder, Invitrogen) in parallel.

2.2.3. Construction of ribosomal 16S gene libraries

Broad range universal primers AF-27 (5′-AGAGTTTGATCMTGG CTCAG-3′) and HR-1492 (5′-TACGGYTACCTTGTTACGACTT-3′) [36] were used for the 16S rDNA amplification. The amplification reactions (done in triplicates) were performed using the following reaction mixture: 1 μL of DNA template, 1 U of AccuPrime™ TaqDNA Polymerase High Fidelity (Invitrogen), 5 μL of 10X AccuPrime™ Buffer II, 5 μL of each primer (20 μM) and ultrapure water (Invitrogen) to a final volume of 50 μL. The optimal annealing condition were determined by gradient PCR in order to maximize specificity and DNA yield. The optimal annealing temperature was 53.9 °C. The reaction was carried out in a thermal cycler (BioRad My Cycler, Bio-Rad Laboratories, Inc.) using the following cycling conditions: an initial step of 94 °C for 60 s, followed by 35 cycles of 94 °C for 30 s, optimized annealing temperature for 10 s and 68 °C for 110 s. PCR products were purified from agarose gels using the method described by Tautz and Renz