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Short communication

Effects of the photochemical transformation of dissolved organic matter on bacterial physiology and diversity in a coastal system

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ABSTRACT

The effect of solar radiation on dissolved organic matter (DOM) and the subsequent impact of photo-altered DOM on bacterial activity and community structure were assessed during two experiments in the coastal system of the Ría de Vigo (NW Iberian Peninsula). After exposure of 0.2 μm filtered seawater for 3.5 days to dark and full sunlight, an inoculum of the bacterial community collected at the same time as the exposed water was added and the mixture was incubated for 4 days in the dark at 15 °C. Changes in bacterial production (BP), diversity (assessed by Fluorescence in situ hybridization) and electron transport system (ETS) activity, dissolved organic carbon (DOC) and nitrogen (DON) and DOM humic-like absorption and fluorescence were followed. The exposure to sunlight had no effect on DOM concentrations while an average (±SD) decrease in DOM humic fluorescence of 45 ± 10% was found. The incubations with photo-altered DOM had lower BP (57 ± 11%), ETS (42 ± 9%) and bacterial carbon demand (BCD) (42 ± 8%) compared with the dark incubations, while bacterial growth efficiency (BGE) was unaffected. This suggests that DOM photo-alteration had a negative effect on bacterial metabolism in the study system. The bacterial growth on irradiated DOM resulted in a significant enrichment of the *Gammaproteobacteria* group compared with the dark control, indicating that solar exposure of DOM led to rapid changes in the bacterial community composition of the Ría de Vigo.

1. Introduction

Dissolved organic matter (DOM) constitutes the main substrate for heterotrophic bacterial growth and respiration in marine systems (Hedges, 2002). DOM in coastal waters can be differentiated according to its origin as autochthonous or allochthonous. Autochthonous DOM is produced within the system and originates largely from phytoplankton exudation, cell lysis, and grazing (Nagata, 2000), whereas allochthonous DOM primarily originates from terrestrial plant and soil materials (Cauwet, 2002).

There is considerable evidence that coloured DOM (CDOM) can be transformed by UV-B (280–320 nm) and UV-A (320–400 nm) solar radiation into reactive oxygen species (ROS) (Scully et al., 2003), inorganic carbon species (CO_2 and CO) (Bertilson and Tranvik, 2000), labile organic and inorganic compounds (Kieber et al., 1989) and further induce the production of biological refractory humic substances (Harvey et al., 1983; Kieber et al., 1997). ROS species may enhance microbial activity, since they can convert biological refractory DOM into labile forms, while they can also lower microbial activity by competing for substrates and causing oxidative stress (Scully et al., 2003; Lesser, 2006). The direct photomineralization of DOC to CO or CO_2 does not provide new bacterial substrate (Bertilson and Tranvik, 2000), while the photochemical conversion of biological refractory DOM into new organic and inorganic compounds may stimulate bacterial growth (Vähatalo et al., 2003). In contrast, sunlight can also induce the biological polymerization and condensation of DOM into refractory materials, representing a source of CDOM (Harvey et al., 1983; Kieber et al., 1997). While some studies suggest that autochthonous DOM gets less and allochthonous DOM more bioavailable after UV exposure, other studies have found the converse (Obernosterer and Herndl, 2000; Tranvik and Bertilson, 2001; Sulzberger and Durisch-Kaiser, 2009).

The bacterial community in marine waters is often dominated by a few major bacterial groups: *Alpha-*, *Beta-* and *Gammaproteobacteria*, and the group *Bacteroidetes* (Giovannoni and Rappé, 2000; Giovannoni and Stingl, 2005). Several studies have shown that the

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DOM composition influences the bacterial community composition (Pérez and Sommaruga, 2006; Teira et al., 2009). However, little is known about the influence of photochemically altered DOM on the bacterial community composition. A recent study by Piccini et al. (2009) in a coastal lagoon suggests that photochemical transformation of DOM stimulates the growth of the *Alpha-* and *Beta-proteobacteria* groups. But this finding needs to be investigated in more detail before we can reach general conclusions. The present study assessed the bacterial community response to irradiated DOM in a coastal system dominated by autochthonous inputs. We hypothesize that photochemically altered DOM will have a negative impact on bacterial activity and change the bacterial community composition.

2. Material and methods

2.1. Sampling site

The Ría de Vigo is a large coastal embayment, with a length of 33 km, a surface area of 176 km^2, and a volume of 3.32 km^3. Its hydrographic regime is dictated by wind-driven upwelling and downwelling episodes; northerly winds result in upwelling, which prevails from April to October, with nutrient-rich upwelled water reaching the surface resulting in a high productivity. From November to March, southerly winds predominate, resulting in downwelling forcing warm and nutrient-poor shelf surface water into the Ría (Álvarez-Salgado et al., 2003). In the winter, the phytoplankton community consists mainly of small phytoplankton species and diatoms which are followed by a spring diatom bloom. During upwelling episodes, phytoplankton primary production reaches a mean value of 2.5 g Cm^{-2} d^{-1}, which decreases to <0.3 g Cm^{-2} d^{-1} during downwelling episodes (see review by Álvarez-Salgado et al., 2010). The bacterial abundance varies seasonally between 0.3 and 2.7 $\times$ 10^9 cells l^{-1} and the bacterial production reaches levels between 2.8 and 66.7 μg C l^{-1} d^{-1} (Lekunberri et al., 2010; Teira et al., 2013). The bacterioplankton community is dominated by the groups *Bacteroidetes* and *Roseobacter* (Teira et al., 2008; Alonso-Gutiérrez et al., 2009), although SAR11 can become occasionally abundant in this eutrophic system (Teira et al., 2009).

Two separate experiments were conducted during winter (14 February 2008) and spring (24 April 2008) with water samples collected in the middle segment of the Ría de Vigo (42° 14.5′N and 8° 45.8′W). Previous studies have shown large differences in DOM concentration and composition between these two periods (Nieto-Cid et al., 2005; Lønborg et al., 2010a). Samples were taken at 5 m depth using a 25 l Niskin bottle. Salinity and temperature were measured prior to the sampling with an SBE 9/11 CTD probe. Samples for chlorophyll *a* (Chl. *a*) were collected by filtering seawater (100–200 ml) through a GF/F filter and analysed after 90% acetone extraction with a Turner Designs 10000R fluorometer (Yentsch and Menzel, 1963).

2.2. Experimental design

Filtration of the collected seawater started within 10 min of collection; one part was filtered through pre-combusted (450 °C for 4 h) GF/C filters to establish a microbial culture, which was kept in the dark at 15 °C until used. The second part was gravity filtrated through a dual-stage (0.8 and 0.2 μm) filter cartridge (Pall-Acropak supor Membrane), which had been pre-washed with Milli-Q water (>10 l).

In the laboratory, the 0.2 μm filtered seawater was divided into two experimental treatments; dark (dark) and full sunlight treatment (UV). The dark treatments were established by placing the 0.2 μm filtered seawater in 500 ml glass bottles covered with aluminium foil and dark plastic bags. The water for the UV treatments was distributed into 270 ml quartz tubes (diameter: 5 cm). No headspace was left in either the glass bottles or the quartz tubes, and all incubators were sealed with ground glass stoppers. The samples were thereafter placed in a recirculation water bath (water depth: 2.5 cm) in the terrace of the laboratory and exposed to 100% natural sunlight for 3.5 days encompassing the natural light/dark cycle. This exposure time was fixed on basis of the decay rate constants of coloured dissolved organic matter previously obtained by Nieto-Cid et al. (2006) in winter (0.25 d^{-1}) and spring (0.40 d^{-1}) in the Ría de Vigo. At these rates, a colour loss of 60–75% would occur in 3.5 days. Before and after sunlight exposure, subsamples were collected for the analysis of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN: NH_4^+, NO_2^-, NO_3^-) and phosphate (DIP: HPO_4^{2-}), and DOM optical properties (absorption and induced fluorescence). Within 30 min after sunlight exposure, the water was combined into two different (dark and UV) carboys and the microbial community collected at the time of sample collection (3.5 days before), was added in a ratio of 1 part of microbial culture to 9 parts of exposed water. The water was thereafter transferred into 500 ml glass bottles and incubated in the dark at a constant temperature of 15 °C, with four replicate bottles being used for sub-sampling at incubation times 0, 1, 2, and 4 days. Unfiltered water from these bottles was used to follow changes in bacterial abundance (BA), production (BP), electron transport system (ETS) activity and diversity using Catalysed Reporter Deposition-Fluorescence In Situ Hybridisation (CARD-FISH). Samples for the analysis of DIN, DIP, DOC, TDN and CDOM absorption were collected in four replicates at day 0 and 4. DOM fluorescence (FDOM) was measured at incubation day 0, 1, 2, and 4. Samples for the dissolved phase were collected after filtration through 0.2 μm filters (Pall Supor membrane Disc) in an acid-cleaned glass filtration system under low N_2 flow pressure. All glassware used was acid washed in 10% HCl and rinsed with Milli-Q and sample water prior to use.

2.3. Sample analysis

BA was determined on day 0, 1 and 2 by fixing the samples (1–2 h) with formol in the dark, filtered onto 0.2 μm polycarbonate filters, and stored at −20 °C until counted. The samples were stained with a DAPI-mix before counted on a Leica DMBL microscope equipped with a 100-W Hg-lamp; more than 800 DAPI-stained cells were counted per sample. Bacterial biomass was calculated from BA, using a carbon conversion factor of 30 fg C $cell^{-1}$, which is representative for coastal bacterial assemblages (Fukuda et al., 1998).

BP was measured by [^{3}H] thymidine incorporation (Fuhrman and Azam, 1980) on days 0, 1 2 and 4. Four replicate 9.9-ml samples and 2 trichloroacetic acid killed samples were added an aqueous stock solution of [^{3}H − methyl] thymidine (40 nmol final concentration). The samples were incubated in the dark at 15 °C for 2 h, 10 ml of ice-cold Trichloracetat (TCA) was thereafter added and samples were filtered onto 0.2 μm polycarbonate filters (presoaked in non-labelled thymidine), washed with 95% ethanol and autoclaved Milli-Q water. The filters were hereafter dried at room temperature (24 h) and mixed with 10 ml of scintillation fluid (Sigma-Flour). The radioactivity incorporated into cells was counted using a Beckman spectral liquid scintillation counter. Thymidine incorporated into bacterial biomass was converted to carbon production using the theoretical conversion factors 2 $\times$ 10^{18} cells mol^{-1} thymidine (Fuhrman and Azam, 1980) and a carbon conversion factor of 30 fg C $cell^{-1}$ (Fukuda et al., 1998).