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Factors shaping bacterial phylogenetic and functional diversity in coastal waters of the NW Mediterranean Sea

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ABSTRACT

To evaluate the main factors shaping bacterioplankton phylogenetic and functional diversity in marine coastal waters, we carried out a two-year study based on a monthly sampling in Blanes Bay (NW Mediterranean). We expected the key factors driving bacterial diversity to be (1) temperature and nutrient concentration, together with chlorophyll *a* concentration as an indicator of phytoplankton biomass and, hence, a carbon source for bacteria (here called bottom-up factors), and (2) top-down pressure (virus- and protist-mediated mortality of bacteria). Phylogenetic diversity was analyzed by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA. Functional diversity was assessed by using monomeric carbon sources in Biolog EcoPlates and by determining the activity of six extracellular enzymes. Our results indicate that the bacterial phylogenetic and functional diversity in this coastal system is shaped mainly by bottom-up factors. A dendrogram analysis of the DGGE banding patterns revealed three main sample clusters. Two clusters differed significantly in temperature, nitrate and chlorophyll *a* concentration, and the third was characterized by the highest losses of bacterial production due to viral lysis detected over the whole study period. Protistan grazing had no effect on bacterial functional diversity, since there were no correlations between protist-mediated mortality (PMM) and extracellular enzyme activities, and utilization of only two out of the 31 carbon sources (N-acetyl-D-glucosamine and α-cyclodextrin) was correlated with PMM. In contrast, virus-mediated mortality correlated with changes in the percentage of use of four carbon sources, and also with specific leu-aminopeptidase and β-glucosidase activity. This suggests that viral lysate provides a pool of labile carbon sources, presumably including amino acids and glucose, which may inhibit proteolytic and glucosidic activity. Our results indicate that bottom-up factors play a more important role than top-down factors (i.e. viral lysis and protistan grazing) in shaping bacterial community structure and activity. Furthermore, they suggest that viruses play a more important role than protists in modifying community structure and functional diversity of bacteria in oligotrophic marine coastal waters.

1. Introduction

Prokaryotes constitute half of the total amount of carbon enclosed in living organisms, and represent the largest pool of nitrogen and phosphorous in the biosphere (Whitman et al., 1998). In aquatic environments, heterotrophic bacteria are an essential part of the food webs, and are the main consumers of dissolved organic matter (DOM) in the ocean (Azam, 1998). The carbon and nutrients contained in bacterial cells can move up along the trophic web through grazing by protists, or return as DOM and particulate organic matter (POM) pools to the water through viral infections and subsequent cell lysis. Thus, viral activity provides an additional input of organic matter and nutrients to ocean waters which, together with the DOM produced by other members of the marine biota, could be available for bacterioplankton (Weitz and Wilhelm, 2012). Although the process of recycling from bacterial cells to the environment can be quantitatively important, little is known of the composition of organic matter released during grazing by protists

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or viral lysis (Weinbauer et al., 2011). For protists, Gruber et al. (2006) found little changes in DOM composition during grazing by ciliates in culture. For viruses, several findings, obtained mainly from virus-host systems, suggest that viral lysis changes the composition of DOM: 1) lysis products have been found to be labile and turn over rapidly (Noble and Fuhrman, 1999), 2) lysis products may be an important source of P for marine bacterioplankton (Weinbauer et al., 1995; Riemann et al., 2009) and 3) DOM released during lysis could be mainly composed of dissolved combined amino acids (51%–86%; Middelboe and Jørgensen, 2006).

DOM in aquatic systems is composed mostly of polymeric compounds and therefore has to undergo hydrolysis before bacterial uptake. Hydrolysis is carried out by specific extracellular enzymes mainly synthetized by bacteria, and their activity is an indirect indication of the polymeric molecules available in the environment (Hoppe, 1983). Monomeric DOM is a preferable carbon source for bacteria, since it can be easily taken up and does not require the synthesis of specific extracellular enzymes. The pattern of utilization of sole carbon sources in Biolog plates (mainly monomers) is characteristic for each community, and has been used to show differences in functional diversity of bacterioplankton in contrasting marine environments (Sala et al., 2005a, 2010), among seasons (Sala et al., 2006), and in depth and temporal patterns (Sala et al., 2008). Products of viral lysis provide an additional input of different types of monomers and polymers to the environment. Preferential use of these monomers might suppress the activity of specific bacterial extracellular enzymes and modify the pattern of sole carbon source utilization by the microbial community.

The structure of natural bacterial assemblages can be shaped by a variety of parameters and processes that can be grouped into environmental (bottom-up) factors and predator pressure (top-down) factors. Several studies have shown temporal changes in the dominance of particular bacterial groups in coastal waters (Pinhassi and Hagström, 2000; Ghiglione et al., 2005) and oceanic waters (Morris et al., 2005). These and other studies (Schauer et al., 2003; Alonso-Sáez et al., 2007) suggest that temperature or substrate availability can shape bacterial diversity, and it has been demonstrated that an addition of specific substrates could induce a succession of bacterial species (Pinhassi et al., 1999). Also, changes in the composition of the phytoplanktonic community, e.g. blooms, can influence the shape of the bacterial community (Pinhassi et al., 2004; Ghiglione et al., 2005). Furthermore, activity of predators, protists and phages can also produce changes in bacterial diversity. Grazing by protists has been shown to impact the taxonomic structure of bacterial communities directly (Šimek et al., 1997), for example by selective grazing (Hahn and Höfle, 1999), and indirectly by providing the substrates for bacterial growth (Caron et al., 1988) or by elimination of competitive strains. Bacteriophages can modify bacterial diversity in a variety of ways, e.g. by lysogenic conversion, transduction, resistance induction, or by release of the lysis products to the environment (Weinbauer and Rassoulzadegan, 2004). It has also been hypothesized that through killing bacteria that win the competition for resources, viruses increase or maintain bacterial diversity in the environment (the 'killing the winner' hypothesis; Thingstad and Lignell, 1997; Thingstad, 2000). However, other studies have shown that viruses cause a reduction in the number of bacterial phylotypes (Schwalbach et al., 2004). Microcosm and field studies have shown a great variability of responses of the bacterial assemblage to the presence of viruses (Hewson and Fuhrman, 2006; Bouvier and del Giorgio, 2007), probably partly due to the different impact of viral lysis on different groups of the same microbial community (Winter et al., 2004). This finding shows that the effect of viruses on bacterioplankton diversity is a complex process that may depend on factors such as the presence of binding sites (porins) on the bacterial cell wall (Lenski, 1988), resistance to viral infection (Weinbauer et al., 2007) and the quality of available DOM (Hewson and Fuhrman, 2006).

The work described herein is part of a two-year study performed in the Microbial Marine Observatory of Blanes Bay, NW Mediterranean. The first part of the study focused on evaluating the bacterial mortality caused by viruses and protists and the second part, presented in this paper, on identifying the main factors shaping phylogenetic and functional bacterial diversity. The evaluation of bacterial mortality was broadly described in Boras et al. (2009). Briefly, viruses and grazers together were responsible for an average of 60% of the loss of bacterial production (BP) and viruses were considered a significant source of mortality in Blanes Bay since they removed an annual average of 12%–32% of BP day^{-1}. Based on these results, the second part of the study aims to identify the main factors shaping phylogenetic bacterial diversity and regulating bacterial utilization of monomeric and polymeric carbon sources in this oligotrophic coastal marine environment. These factors were grouped into bottom-up factors (those that derive from supply resources or physical factors: temperature, nutrient concentration, Chl *a* and Chl *a* <3 μm); and top-down factors (those that derive from predator biomass and activity: viral and protist abundances and their bacteria-mediated mortality).

The main aims of the present study were (1) to monitor changes in bacterial community structure during the year, (2) to detect the main factors determining bacterial phylogenetic and functional diversity, the latter based on extracellular enzyme activity and utilization of sole carbon sources, and (3) to evaluate the role of viruses and protists in shaping bacterial phylogenetic and functional diversity. We expected to find (1) changes in bacterial phylogenetic diversity caused by viral lysis and/or protistan grazing, (2) lower specific extracellular enzyme activities with higher viral mortality due to the release of DOM, including monomers during lysis, and (3) higher variability in the utilization of monomeric carbon sources with higher virus-mediated mortality than with protist-mediated mortality since viral lysis releases higher amounts of bacterial DOM.

2. Material and methods

2.1. Study site and sampling strategy

Surface water samples (0.5 m depth) were collected from May 2005 to April 2007 in Blanes Bay, Spain (the Blanes Bay Microbial Observatory, NW Mediterranean, 41°40′N, 2°48′E, 20 m depth), where microbial communities have been investigated for over a decade (Gasol et al., 2012). Samples were collected in 10-L polyethylene carboys once a month, 0.5 miles off the shore, and kept in the dark until they reached the lab (~2 h). Water temperature and salinity were measured in situ with a conductivity, temperature and depth (CTD) profiler.

2.2. Physicochemical and biological parameters

A detailed description of the determination of physicochemical and biological parameters is presented in Boras et al. (2009). Briefly, concentrations of inorganic nutrients (PO_4^{-3} and NO_3), chlorophyll *a* (Chl *a*), and the Chl *a* fraction smaller than 3 μm (Chl *a* <3 μm) were determined using standard methods (Grasshoff et al., 1983 for inorganic nutrients; Yentsch and Menzel, 1963 for Chl *a*). Viral abundances were determined by flow cytometry as described in Brussaard (2004). Bacterial and heterotrophic nanoflagellate (HNF) abundances were obtained by epifluorescence microscopy (Olympus BX40) after staining with DAPI (Porter and Feig, 1980;