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Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters☆

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ABSTRACT

Plastic pollution is widespread in ocean ecosystems worldwide, but it is unknown if plastic offers a unique habitat for bacteria compared to communities in the water column and attached to naturally-occurring organic particles. The large set of samples taken during the *Tara*-Mediterranean expedition revealed for the first time a clear niche partitioning between free-living (FL), organic particle-attached (PA) and the recently introduced plastic marine debris (PMD). Bacterial counts in PMD presented higher cell enrichment factors than generally observed for PA fraction, when compared to FL bacteria in the surrounding waters. Taxonomic diversity was also higher in the PMD communities, where higher evenness indicated a favorable environment for a very large number of species. *Cyanobacteria* were particularly overrepresented in PMD, together with essential functions for biofilm formation and maturation. The community distinction between the three habitats was consistent across the large-scale sampling in the Western Mediterranean basin. 'Plastic specific bacteria' recovered only on the PMD represented half of the OTUs, thus forming a distinct habitat that should be further considered for understanding microbial biodiversity in changing marine ecosystems.

1. Introduction

Plastic litter is the most common form of marine anthropogenic debris and a growing global pollution concern, affecting marine ecosystems and economic activities (Gregory, 2009). The discovery of five large-scale accumulation areas of floating plastic marine debris (PMD) in the subtropical ocean gyres has captured world-wide attention, which often refers to these zones as "great garbage patches" (Cózar et al., 2014; Eriksen et al., 2014). These floating accumulation areas are dominated by plastic pieces, mainly smaller than 5 mm, commonly referred to as "microplastics" (Andrady, 2011; Law and Thompson, 2014). The Mediterranean Sea is another accumulation region with average concentration of more than 200 000 items km^{-2}, comparable to subtropical oceans gyres (Lebreton et al., 2012; Pedrotti et al., 2016). The accumulation of floating plastic in the Mediterranean Sea is explained by the high human density and the hydrodynamics of this semi-enclosed basin with residence time of 70 years (Durrieu de Madron et al., 2011).

Bacterioplankton represent the most abundant organisms in seawater where they are critical for nutrient and carbon cycling, which supplies microbial food webs and higher trophic levels (Gasol et al., 2008). Dissolution and degradation of 75% of the organic particles sinking to the sea floor revealed the key role played by particle attached (PA) bacteria in the regulation of the carbon storage by the Oceans (Farooq and Malfatti, 2007; Mével et al., 2008). Particulate organic carbon and more precisely "phycospheres" and "detritospheres" represent micro-patches of concentrated substrates that are hot spots for pelagic bacterial processes (Ghiglione et al., 2009). PA bacteria differ from the

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surrounding free-living (FL) fraction in many aspects, including cell abundance, morphology, diversity and metabolic activities (Pulido-Villena et al., 2014; Simon et al., 2002). Microplastics may be considered as artificial particles recently introduced within the last 60 years in the Oceans, which offer novel habitats for bacteria and enhance their dispersal over long ways by drifting with currents and winds (Lebreton et al., 2012). Recently, Zettler et al. (2013) introduced the term "plastisphere" to describe the very diverse PMD-associated microorganisms, which were found in the seawater sub-surface to differ from the surrounding FL bacteria. Seafloor plastisphere was also found to be genetically unique from the FL water-column bacteria and in addition, they were distinct from the sediment communities in the North Sea (De Tender et al., 2015).

The purpose of this study was to test the hypothesis that plastic selects for a unique microbial communities compared to PA and FL habitats. This question is not trivial since any surfaces exposed to seawater are rapidly colonized by bacteria and generally form biofilms (Siboni et al., 2007; Lobelle and Cunliffe, 2011). Because the few studies conducted so far on floating microplastics collected at sea only compared the PMD to FL bacteria (Zettler et al., 2013, 2015), the question of the existence of a 'plastic-specific' community in contrast to the PA fraction is still open. Here, we provide microscopic counts and high-throughput, next-generation sequencing of 16S rRNA genes of bacteria living in the PMD, PA and FL fractions collected during the *Tara*-Mediterranean expedition. We also used a computational approach to predict the potential functional differences between the three communities. Finally, we investigated the influence of environmental factors and plastic-related properties as potential drivers of the plastisphere characteristics.

2. Materials and methods

2.1. Plastic and surrounding seawater sampling

Samples were taken during the *Tara*-Mediterranean expedition (April-November 2014) aboard the RV *Tara*. PMD items were recovered from 32 manta trawls (mesh size 333 μm) carried out in the Western Mediterranean Sea (Fig. 1). At each sampling point, a subset of these fragments was randomly sorted with sterilized forceps, rinsed with a wash bottle of sterile seawater and immediately frozen for further physical, chemical and bacterial diversity analysis. For microscopic observations, PMD were fixed for at least 20 min at room temperature with 2% (v/v) glutaraldehyde (final concentration) before freezing. A conductivity-temperature-fluorescence-depth profiler (CTD – SeaBird SBE 19) was deployed at each of the 32 stations, together with Niskin bottle for surface seawater collection. A fractionation procedure was performed onboard in one step for 2 L seawater sequential filtration through 3 μm-pore size (PA fraction) and 0.2 μm-pore size (FL fraction) polycarbonate filters (47 mm diameter, Nucleopore) using a peristaltic pump (pressure <100 mbar) and filters were subsequently frozen until further DNA extraction, as previously described (Ghiglione et al., 1999).

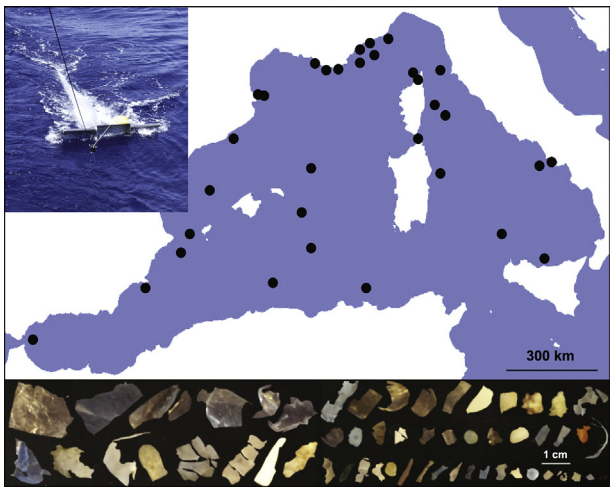

Fig. 1. Study area and station locations (n = 32) in the Western Mediterranean Sea. Upper left: picture of the manta trawls used to collect PMD. At the bottom: light-micrograph of PMD analyzed in this study.

2.2. Physical and chemical characterization of the PMD

The one-dimensional area of the surface, the major length and circularity of the individual PMD were determined using ZooScan system (Hydroptic Ltd., EMBRC France) (Gorsky et al., 2010). Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed on the individual PMD using Spectrum 100 equipped with an ATR attenuated total reflectance (Perkin-Elmer) and compared to references for identification of the nature of the polymer. Carbonyl index values were determined as the ratio of the peak intensity at 1715 cm^{-1} to the peak intensity at 1460 cm^{-1}, as previously described (Selke et al., 2015).

2.3. Microscopic observations

Bacteria were enumerated on 70 plastic pieces sampled at 22 stations (from 1 to 4 pieces per manta, when microscopic observation was possible) by epifluorescence microscopy using an Olympus AX-70 PROVIS after 4', 6-diamidino-2-phenylindole (DAPI) staining. The covering of the surface area of the plastic by bacterial cells was determined using Image J software.

For qualitative assessment of biofilm structure, a random collection of 8 samples was chosen for scanning electron microscopy (SEM) using Inspect S50 (FEI, Hillsboro, OR, USA), as described previously (Zettler et al., 2013). Some of the samples were also analyzed by atomic force microscope (AFM) to get more resolved insight of the surface, using a Nanoscope V in dynamic mode (Bruker instruments, Madisson, WI, USA) and standard silicon probes (Bruker, TESP-V2) (Binnig and Quate, 1986).

2.4. DNA extraction and next-generation sequencing

DNA was extracted from 72 randomly sorted PMD from each the 32 stations and from all the 3 μm- and 0.2 μm-pore size filters from surrounding seawater. Between 2 and 4 plastic fragments (filament, pellet or sheet, size > 2 mm) were extracted separately at each station. DNA extraction followed a classical phenol-chloroform-based protocol (Ghiglione et al., 1999) with an additional sonication step (3 × 5 s with 30% amplitude, Branson SLPe) for a better disruption of the biofilm. The molecular size and the purity of the DNA extracts were analyzed by agarose gel electrophoresis (1%) and the DNA was quantified by spectrophotometry (GeneQuant II, Pharmacia Biotech). Primers for PCR amplification of the 16S V3–V5 region were 515F-Y and 926R, particularly well-suited for marine samples according to Parada et al. (2016), with Illumina-specific primers and barcodes. Sequencing was performed on an Illumina MiSeq by Research and Testing Laboratories (Lubbock, TX). Raw FASTA files were deposited at GenBank under the accession number PRJNA380761. Paired raw reads were joined, quality-filtered and assigned to taxa using Qiime pipelines, as previously described (Severin et al., 2016). Operational taxonomic units (OTUs)