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Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary

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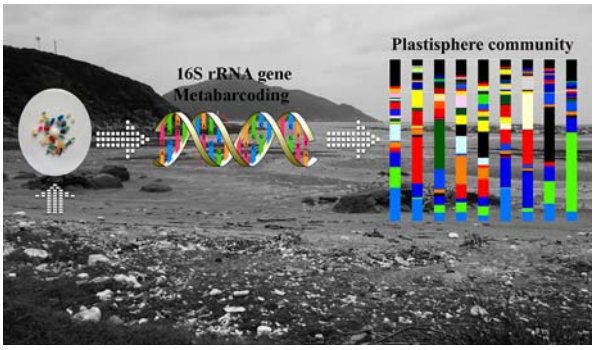
HIGHLIGHTS

- The information of plastic-associated microbial community in the intertidal zone is scanty.
- Microbial communities attached plastics discriminates among three intertidal ecosystems around the Yangtze estuary, China.
- Keystone species were Alphaproteobacteria, Gammaproteobacteria, Flavobacteriia, Acidobacteria and Cyanobacteria.
- Putatively pathogenic species acts as hitchhikers on microplastic particles.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic trash is common in oceans. Terrestrial and marine ecosystem interactions occur in the intertidal zone where accumulation of plastic frequently occurs. However, knowledge of the plastic-associated microbial community (the plastisphere) in the intertidal zone is scanty. We used high-throughput sequencing to profile the bacterial communities attached to microplastic samples from intertidal locations around the Yangtze estuary in China. The structure and composition of plastisphere communities varied significantly among the locations. We found the taxonomic composition on microplastic samples was related to their sedimentary and aquatic origins. Correlation network analysis was used to identify keystone bacterial genera (e.g. Rhodobacterales, Sphingomonadales and Rhizobiales), which represented important microbial associations within the plastisphere community. Other species (i.e. potential pathogens) were considered as hitchhikers in the plastic attached microbial communities. Metabolic pathway analysis suggested adaptations of these bacterial assemblages to the plastic surface-colonization lifestyle. These adaptations included reduced "cell motility" and greater "xenobiotics biodegradation and metabolism." The findings illustrate the diverse microbial assemblages that occur on microplastic and increase our understanding of plastisphere ecology.

1. Introduction

Worldwide plastics production was 322 million tons in 2015 (Plastics Europe, 2016). Improper plastic disposal has led to an accumulation of plastic marine debris in the oceans of the world (Cózar et al., 2014). Plastic litter has been ranked as a new addition to the list of global threats, including climate change, ocean acid and ozone depletion

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(Amaral-Zettler et al., 2015). Plastic marine debris fragments into smaller particles (microplastic, <5 mm) (Thompson et al., 2004). Deleterious effects of plastic debris on marine organisms, such as the physical and chemical effects of entanglement and ingestion by a range of marine organisms from zooplankton to fish, seabirds, sea turtles and cetaceans, have been documented (Gall and Thompson, 2015). Microplastic (3.0 or 9.6 μm) ingested by mussels translocated from the gut to the circulatory system (Browne et al., 2008). Microplastic inside cells can cause significant impacts at the tissue and cellular level, and interfere with energy reallocation, reproductive success, and offspring performance (Moos et al., 2012; Sussarellu et al., 2016).

Microplastic can be colonized by various marine organisms, that may affect the fate and ecological impact of plastic pollution (Kiessling et al., 2015). Due to its light weight and persistence, plastic debris transported by ocean currents and winds may transfer organisms to non-native habitats, posing a threat to biodiversity and coastal environments (Barnes, 2002; Gregory, 2009). Plastic debris lasts much longer than most natural floating substrates such as macroalgae, feathers, or wood, and represents a novel type of pelagic substrate for microbial colonization and transportation (Zettler et al., 2013). Zettler et al. (2013) characterized epiplastic microbial communities (termed the 'plastisphere') from the North Atlantic ocean using 16S metabarcoding. They found that the taxonomic composition of microbial communities on plastic particles was distinct from the surrounding seawater. These plastic inhabitants could be capable of degrading plastic polymers (Zettler et al., 2013; Reisser et al., 2014), altering the buoyancy of polymers (Ye and Andrady, 1991; Lobelle and Cunliffe, 2011), and affecting the toxicity of plastics (Pham et al., 2012; Zettler et al., 2013). The oceanic interactions between microorganisms and plastics are of significant ecological concern. Microbial assemblages associated with plastics differ in relation to the polymers in the plastic substrate, the geographical location, and the sampling date (Oberbeckmann et al., 2014; Amaral-Zettler et al., 2015; De Tender et al., 2015). The metagenomic approach was used for determining the genes expressed in the plastisphere from the North Pacific Subtropical Gyre. The results demonstrated that the microbial populations on microplastics have lifestyles, metabolic pathways, and biogeochemical activities that differ from those of microbes in the surrounding seawater (Bryant et al., 2016; Debroas et al., 2017).

Due to the ecological importance of the plastisphere, additional study of the plastic-colonizing community in different environments may provide information useful for mitigation of plastic debris. Serving as the hydrographic link between anthropogenic activities in the upland and the adjacent marine environments makes the intertidal zone become a hot spot for plastic debris accumulation, which has been documented by previous studies (Aguilera et al., 2016; Browne et al., 2010). Previous studies of the ocean plastisphere focused on floating plastic litter on the water surface. Most plastic debris accumulates in the sediment, particularly in the coastal zones (Harrison et al., 2014). However, knowledge of the microbial community attached to plastic marine debris in the sediment environment is limited (De Tender et al., 2015). We used next-generation amplicon sequencing to study the bacterial community on microplastics collected in the intertidal zone of the Yangtze estuary, China. Our aims were to study the: 1) plastic-associated microbial communities in the intertidal zone; 2) patterns in plastic-associated community structure related to geographic locations and 3) key bacterial groups and their corresponding functionality in plastic attached communities.

2. Materials and methods

2.1. Sample collection

Microplastic samples were collected at three stations in April 2016: Xiangshan Bay (29°30′29.9″N, 121°27′27.3″E), Chongming Island (31°36′57.9″N 121°23′30.0″E) and Lvsi Port (32°04′52.4″N 121°36′10.1″E) (Fig. S1). Chongming Island is located in the Yangtze estuary and is strongly influenced by the freshwater from the Yangtze River. During low tide, a total of thirty four microplastics stranded on the surface of the muddy intertidal areas were collected with sterile forceps. Microplastics were placed into sterile 15 ml plastic tubes, immediately stored on ice, and transported to the laboratory where the samples were stored at −20 °C until analysis.

2.2. Spectroscopic analysis

The polymeric matrix of plastic pieces was studied using micro-FTIR spectroscopy (BRUKER LUMOS). Two replicate FTIR spectra were acquired on different spots of each PMD piece. All spectra were recorded with the average of 32 scans, at 4 cm^{-1} resolution. Spectra processing and analysis were done according to a recent plastic identification protocol (Zhao et al., 2017). Each measured FTIR spectra was compared with spectra in a commercial library (BioRad-KnowItAll Informatics System, Thermo Fisher Scientific Inc.).

2.3. DNA extraction and sequencing

DNA in plastic samples was extracted using MoBio Powersoil DNA extraction kits (MoBio Laboratories, Carlsbad, CA) following manufacturer instructions. The V3-V4 variable region of the 16S rRNA genes was amplified using the primer sets of 319F (5′-ACTCCTACGG GAGG CAGCAG-3′) and 806R (5′-GGACTACHV GGGTWTCTAAT-3′). PCR amplification was conducted as follows: initial denaturation at 98 °C for 30 s; 35 cycles of 98 °C, 10 s; 54 °C, 30 s; and 72 °C, 45 s, and a final extension step at 72 °C for 10 min. The 25 μl PCR master mixes contained 50 ng of template DNA, 12.5 μl of the Premix Ex Taq™ hot start version, and 0.1 mM of each primer. PCR products were purified using beads. The resulting amplicons were pyrosequenced using Illumina MiSeq v3 technology (2 × 350 bp, paired-end) by LC SCIENCES, Hangzhou, China. DNA samples (3 DNA samples × 3 sampling sites) were successfully assessed from 9 (4.61 ± 0.27 mm) of the 34 microplastic particles collected from the 3 locations, and used for further high-throughput sequencing. The raw sequence reads were deposited into the NCBI sequencing read archive under Accession No SRP125152.

2.4. Sequencing data treatment

Raw sequences were processed using MOTHUR v.1.33.3 software (Schloss et al., 2009). Briefly, barcode and adapter primer sequences were clipped off the reads. Paired reads were assembled. The sequences met the following criteria: (1) the sequence matches the 806R primer and one of the used barcode sequences; (2) no ambiguous bases were found within the sequence; (3) the sequence had a length of ≥200 bp; (4) the sequence had an average quality score ≥25; (5) homopolymers in the sequence were <8 bp. Sequences containing an N (undetermined nucleotides) ratio >5% of the sequence and generating low-quality value (Q) reads (the base number of Q < 10 >20% in the entire read) were removed from the data set.

2.5. Data analysis

CD-HIT was used for clustering raw sequences into operational taxonomic units (OTUs) at a 97% similarity level. The OTU statistics and graphical output were conducted in R program version 3.3.1. The longest sequence in each category was designated as the representative OTU sequence which was annotated by RDP, Greengenes and the NCBI 16S Microbial database. Alpha Diversity was calculated in QIIME v1.3.0. PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states), encompasses data from the United States Department of Energy Joint Genomic Institute's Integrated Microbial Genomes (IMG) database (Markowitz et al., 2012). PICRUSt was utilized to normalize the 16S copy number for each OTU and predict the