Front. Environ. Sci. Eng. 2022, 16(11): 145
https://doi.org/10.1007/s11783-022-1580-1

RESEARCH ARTICLE

Plastic materials and water sources actively select and shape wastewater plastispheres over time

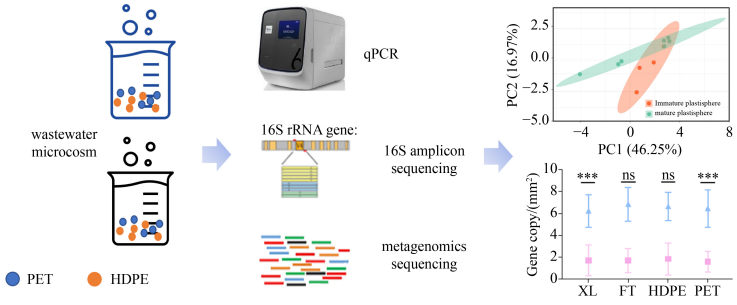
Yu Xia[1,2,3,#], Xuyang Zhang[1,#], Miao Zhang[1,#], Liming Chen[1], Xiaotong Tang[1], Yuhong Sun[1], Xiang Li (✉)[1,2,3]

1 School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China
2 State Environmental Protection Key Laboratory of Integrated Surface Water-Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China
3 Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China

HIGHLIGHTS

- Wastewater MPs exhibited resistomes and therefore health threats.
- High density of alkB gene indicates both HDPE and PET can be utilized by microbes.
- Plastics and waters actively selected and shaped the plastispheres over time.
- A broader phylogenetic spectrum of MHET-degrading microorganisms was annotated.

GRAPHIC ABSTRACT



ARTICLE INFO

Article history:
Received 23 January 2022
Revised 19 April 2022
Accepted 23 April 2022
Available online 13 June 2022

Keywords:
Microplastics
Whole-genome metagenomic sequencing
Plastisphere
Plastic degradation
MHETase

ABSTRACT

The daily use of plastics presents a serious pollution issue due to their extremely slow degradation. Microplastics and the biofilm that grows on plastics (i.e., the plastisphere) are important subsets of plastic wastes. Many studies have been conducted to reveal the structures of the plastispheres, the driving factors for the formation of the plastisphere, and the ability of the plastispheres to degrade plastics in a variety of water bodies. However, the plastispheres related to wastewater are understudied. In this study, we used a microcosmic strategy to study the evolution of the plastispheres associated with microplastics (MPs) over time in wastewater. We found that plastic materials and water sources did not actively select and shape the plastispheres at an early stage, but the active selection for a unique niche of the plastisphere occurred after 14 d of growth. In addition, we confirmed that the *alkB* gene was densely present, and metagenomics showed some additional chemical reactions, which suggests that MPs are consumed by the microbes in the plastispheres. Additionally, metagenomics identified some metagenome-assembled genomes (MAGs) associated with high-density polyethylene (HDPE) and polyethylene terephthalate (PET). The identification of HDPE-associated MAGs and PET-associated MAGs further supports the notion that the selection for a unique niche of the plastisphere is driven by plastic materials and water sources (in this study, after 14 d of growth). Our discoveries bring new views on the behavior of the wastewater-associated plastisphere, especially how long it takes a wastewater plastisphere to form.

1 Introduction

Plastics, which are important man-made consumables, are composed of organic compounds and present a serious pollution problem to the environment due to their extremely slow degradation. Unfortunately, discarded plastics may enter into aquatic ecosystems such as seas (Zettler et al., 2013), rivers (Lechner et al., 2014), and lakes (Eriksen et al., 2013), from not only effluents of wastewater treatment plants (Estahbanati and Fahrenfeld, 2016), but also surface runoff, rain, and storms. Microplastics (MPs), which are plastic wastes that raise

✉ Corresponding author
E-mail: lixiang.henry@gmail.com

# These authors contributed equally to this work.

great concerns, are plastic particles whose diameter is smaller than 5 mm. Some studies have shown that the concentration of MPs in effluents of wastewater treatment plants (WWTPs) significantly increases and can be transferred to downstream receiving waters (Estahbanati and Fahrenfeld, 2016; Li et al., 2018; Kazour et al., 2019). MPs accumulate a diverse microbial community called the plastisphere (Zettler et al., 2013). Notably, Pham et al. (2021) found that the presence of MPs in wastewater can enrich antibiotic-resistant bacteria and pathogenic bacteria because MPs can serve as substrates for the bacteria (Pham et al., 2021). After being released onto the water surface, the bacteria can spread quickly. However, research on the driving factors for the formation of the plastisphere and changes in the plastisphere over time is lacking.

Does the plastisphere always share a similar composition? Recently, a growing body of evidence has suggested that the structure of the microbial community is distinctly diverse, and the driving factors for the formation of the plastisphere include not only the type of the material (Zettler et al., 2013; Ogonowski et al., 2016), biomass in the plastisphere (Zettler et al., 2013; McCormick et al., 2014; Ogonowski et al., 2016), the abundance of primary and secondary colonizers (De Tender et al., 2015), age of plastics (Harrison et al., 2014), and location of sampling (Harrison et al., 2014; McCormick et al., 2014; Oberbeckmann et al., 2016), but also environmental factors such as weather, salinity, and nutrients (Ogonowski et al., 2016). Amplicon sequencing methods have been used intensively to study the profile of the plastisphere. However, there are only a few studies based on the metagenomic analysis of the plastisphere (Pinnell and Turner, 2019; Zhao et al., 2020; Bhagwat et al., 2021). Researchers can use metagenomic analysis to interpret metabolic activities and understand how the plastisphere uses plastics as energy sources. Evidence based on metagenomic analysis is critical to engineering the enzymes related to the degradation of plastics (Yoshida et al., 2016; Son et al., 2019) for alleviating plastic pollution.

Polyethylene (Ogonowski et al., 2016) and its derivatives such as low-density polyethylene (Harrison et al., 2014; Pinto et al., 2020), high-density polyethylene (Lagarde et al., 2016), and polyethylene terephthalate (Oberbeckmann et al., 2016) are plastic materials whose biodegradation has been widely studied because they are used extensively in daily life (e.g., in water bottles and supermarket bags). Researchers have tried to link the characteristics of polyethylene (PE)-associated microbes to the physiochemical characteristics of the plastic (Duan et al., 2021). For instance, Ogonowski et al. (2016) found that there are no significant correlations between the density of biofilms and the hydrophobicity of substrates; however, the structure of the microbial community is strongly linked to the hydrophobicity of the substrate. Another important finding is that the characteristics of substrates affect the composition rather than the abundance of the bacterial community (Ogonowski et al., 2016). Moreover, Pinto et al. (2020) explored the plastic-degrading ability of the prokaryotes in a marine environment and revealed that 35 operational taxonomic units (OTUs) can be enriched in the laboratory. As low-density polyethylene (LDPE) was their sole carbon source, the microbes were classified as putative LDPE degraders. Furthermore, by using sequencing and microspectroscopic analyses, the authors proved that some of the OTUs are related to alkane degraders and hydrocarbon degraders (Pinto et al., 2020).

In this study, we exposed effluents of WWTPs to spherical HDPE and PET particles under controlled conditions (a microcosmic experiment) for two weeks. The main water sources for the plastisphere originated from the Xili municipal WWTP (XL), China, which is located at 22.966°N and 114.593°E, and Futian municipal WWTP (FT), China, which is located at 22.535°N and 114.018°E. River and marine ecosystems were the receivers of the effluents. In this study, qPCR, amplicon sequencing, and whole-genome metagenomic sequencing (hereafter abbreviated as metagenomic sequencing) were used to understand 1) the influence of plastic materials and water sources on the structure of the plastisphere over time; and 2) the evidence of the degradation of plastics.

2 Materials and methods

2.1 Setup of microcosmic experiments, determination of sampling methods, and extraction of DNA

Before microcosmic experiments were conducted, commercial spherical HDPE and PET particles had been sieved into 3-mm or 5-mm particles. The particles were autoclaved and then placed in sterilized gauze bags before being incubated with effluents from 2 WWTPs (Table 1). The effluent from FT was collected from the marine environment in which the effluent was discharged, and the effluent from XL was collected from a riverine environment. In this study, the microcosmic experiment was performed continuously for 14 d, and 5 sampling events (3 h, 8 h, 1 d, 6 d, and 14 d) were performed during the experiment. Sixty eligible samples were obtained after the barcode screening of 16S rRNA amplicon sequencing (see the Supplementary file). All of the samples were collected aseptically and stored at 4 °C before DNA extraction within 24 h. The FastDNA kit (MP Biomedicals, USA) was used for DNA extraction by following the manufacturer's instructions.

2.2 qPCR analysis

The V3 and V5 regions of the 16S rRNA gene were quantified using the primers published by Lee et al.

Table 1 Parameters of effluents discharged from studied wastewater treatment plants

Parameters	Effluent parameters (mg/L) (average values)	
	XL	FT
pH	7.04	6.81
SS	5	5
BOD5	1.5	2.1
COD	17	14
Total P	0.11	0.12
Ammonia-N	0.31	0.59
NO_3-N	6.52	6.53
NO_2-N	2.260	0.055
Total N	10.60	8.36
TDS	301	618
TS	307	633

(2008), and the alkane hydroxylase gene (the *alkB* gene) was quantified using a published *alkB* primer (forward: 5'-TCGAGCACATCCGCGCCACCA-3', reverse:5'-CC-GTAGTGCTCGACGTAGTT-3'). qPCR analysis for the 16S rRNA gene was performed using the Applied Biosystems Quant Studio 5 Real-Time PCR System (Thermo Fisher Scientific, USA). We used TB Green Fast qPCR Mix (TaKaRa, Japan) to conduct qPCR cycling for V3 and V5 regions. The reaction mixture contained 5 μL of TB Green (TaKaRa, Japan) added with 0.2 μL of Rox II, 0.4 μL of the BAC338F primer (10 μm), 0.4 μL of the BAC805R primer (10 μm), 1 μL of the DNA template, and 3 μL of H_2O. The total volume of the mixture was 10 μL. The initial denaturation was performed at 95 °C for 30 seconds, which was followed by 35 cycles of denaturation at 95 °C for 5 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 60 seconds. The QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, USA) and 2 × SYBR Green Realtime PCR Mix (Bioteke Corporation, China) were used to quantify the *alkB* gene. The reaction mixture contained 12.5 μL of the 2 × PCR mixture added with 1 μL of the forward primer (10 μm), 1 μL of the reverse primer (10 μm), 1 μL of the DNA template, and 9.5 μL of the water added with the ROX II dye. The total volume of the reaction mixture was 25 μL. The initial denaturation was performed at 94 °C for 2 minutes, which was followed by 40 cycles of the denaturation at 94 °C for 15 seconds and annealing at 60 °C for 30 seconds. All qPCR curves exhibited one additional dissociation step (the annealing at 60 °C for 1 min). For qPCR analysis, the Cq threshold was manually set to 0.01, and the corresponding data were analyzed using QuantStudio™ Design & Analysis Software (Thermal Fisher, USA) and Microsoft Excel. In this study, a sample was considered to contain the *alkB* gene when 2 out of 3 qPCR reactions were amplified. In addition, for each qPCR reaction, 6 no-template controls (NTCs) were incorporated into the screening for exogenous DNA contamination.

2.3 16S rRNA amplicon sequencing

The primers for the V3 and V4 regions of the 16S rRNA gene (primers 341F and 805R) were used for amplicon sequencing by using designated barcodes (Klindworth et al., 2013). The Premix Taq reagent (Taq V2 and dyes; TaKaRa, Japan) was used to perform PCR reactions by using the BioRad T100 Thermal Cycler (BioRad, USA). The 50-μL reaction mixture contained 25 μL of the Premix Taq reagent, 1 μL (10 μm) of the 341F primer, 1 μL (10 μm) of the 805R primer, 1 μL of the DNA template, and 22 μL of water. The PCR amplification involved 30 cycles of denaturation at 94 °C for 30 sec., annealing at 55 °C for 30 sec., and extension at 72 °C for 60 sec. After PCR amplification, unnecessary fragments and chemicals were removed by using the PureLink Quick PCR Purification Kit (QIAGEN, USA). Purified DNA samples were sent to Majorbio (Shanghai, China) for amplicon sequencing using the Miseq platform and PE300 strategy.

2.4 Whole-genome metagenomic sequencing

Due to the limited availability of DNA samples, we combined 6 DNA samples to generate 1 DNA mixture for metagenomic sequencing. Four metagenomic samples that target XL, FT, HDPE, and PET were sequenced (see the Supplementary file). In this study, we performed metagenomic sequencing using the Illumina HiseqXten-PE150 platform at Novogene (Nanjing, China). The size of the sequencing library was 350 bp and 10-G raw data were targeted.

2.5 Processing and analysis of data obtained by using next-generation sequencing (NGS)

We used fastp to check the quality of amplicon-sequencing data and metagenomic-sequencing data. Low-quality data were discarded before further analysis. For amplicon sequencing, the fastq-join program (Aronesty, 2013) was used to merge paired-end reads and extract barcodes. Cutadapt was used to trim the primers (-g CCTACGGGNBGCASCAG -a GGATTAGATACCCBN-GTAGTC -e 0.15). Then, OTUs were clustered on the basis of a 97% similarity threshold, and the taxonomic groups of the OTUs were assigned using the ribosomal database project. Finally, QIIME1 was used for OTU-related statistical analysis. Moreover, we identified the concentrations of normalized ARGs in samples and compared the concentrations of the ARGs among the samples by using ARGs-OAP v2.0 (Yin et al., 2018). The metagenomic samples were binned using the metaWRAP framework and its default settings (combining binning results obtained by using MetaBAT (Kang et al., 2019),

MaxBin2 (Wu et al., 2016), and CONCOCT). CheckM was used to check the completeness and level of contamination of metagenome-assembled genomes. High-quality metagenome-assembled genomes (MAGs) whose completeness was above 50% and level of contamination was below 10% were used for subsequent functional analysis. After binning, metabolic and biogeochemical profiles were predicted using the default setup of METABOLIC software. Moreover, prodigal was used to predict open read frames (ORFs) from metagenomic assemblies, and the predicted ORFs (> 1 kb in length) were aligned against the Swiss-Prot database. KEGG orthology (KO) numbers were obtained by using Prokka (Seemann, 2014). The KO numbers were imported to KEGG Mapper by using the "reference search mode", and the pathways related to the degradation of plastics were summarized. The Sankey plot was used to determine the most abundant pathways related to the degradation of organic compounds and the bacterial hosts of the pathways. In maximum likelihood phylogenetic analysis of MHETase, the built-in MUSCLE option in MEGA software (Kumar et al., 2018) was used to align protein sequences using the UPGMA clustering method, and then evolutionary history was inferred using the maximum likelihood method and Whelan and Goldman model. The tree that had the highest log likelihood (−37957.61) was shown. Percentages of trees in which associated taxa were clustered were shown next to branches. Scales were used to draw the trees, and the length of branches was expressed in terms of the number of substitutions. The whole analysis involved 46 amino acid sequences, and 823 positions were included in the final dataset. Moreover, we aligned protein sequences to the Pfam database, and the active sites predicted using the database and matched domains were annotated.

2.6 Calculation and statistical analysis

For qPCR calculations, we estimated the surface area of spherical particles to normalize the concentration of the gene copy per mm^2. Then, student's t tests (two-tailed, heteroscedasticity hypothesis) were performed to search for differences in the concentrations of the *alkB* and 16S rRNA genes among different groups ($p < 0.05$). Principal component analysis (PCA) was performed to cluster the biofilms using the "hellinger" method. The relative abundance of each of the 39 amplicon samples was calculated. After amplicon barcoding, 39 samples were used for PCA (OTU numbers > 800). A heatmap was used to analyze the 39 samples using the "centroid" clustering in R (the "heatmap" function; for each sample, OTUs were normalized). In this study, three grouping strategies were applied: samples were grouped on the basis of "plastic materials", "water sources", or a combination of "plastic materials", "water sources", and "incubation periods". The "vegan" package in R was used for visualization. The "anosim" function in the "vegan" package was used to confirm the statistical significance of differences among those groups. Finally, Microsoft Excel was used to provide the results of temporal relative abundance. The average of the relative abundance was greater than 1%.

3 Results and discussion

3.1 Summarization and availability of NGS data

In this study, 155946 16S amplicon reads were obtained after quality checking. Additionally, the HDPE, PET, XL, and FT metagenomic samples generated 16114854; 18041580; 18816498; and 19758848 quality-checked reads, respectively (0.03 error rates, Q30 > 93.28%). Our sequencing data were deposited in the Bio-Med Big Data Center (BMDC). The accession numbers of the amplicons are SUB006766, SUB006805, SUB006806, SUB006807, SUB006809-13, and SUB006816-22. The accession numbers of the metagenomic samples are SUB006798, SUB006799, SUB006802, and SUB006808.

3.2 Threats of the antibiotic resistant plastisphere

As shown in Table 2 (see the Supplementary file for sample information), 15 types of antibiotic resistance were detected, among which the multidrug resistant type exhibits the highest concentration, followed by the bacitracin resistant type and beta-lactam resistant type. The concentrations of ARGs in XL are almost two times as high as those in FT. Both HDPE and PET exhibit

Table 2 Concentrations of ARGs (gene copies/cell) in samples of the plastisphere

Antibiotics	FT	XL	HDPE	PET
Aminoglycoside	0.006	0.018	0.019	0.017
Bacitracin	0.075	0.176	0.137	0.144
Beta-lactam	0.011	0.059	0.111	0.090
Chloramphenicol	0.008	0.003	0.002	0.003
Fosmidomycin	0.004	0.026	0.020	0.025
Kasugamycin	0.003	0.001	0.001	0.001
Macrolides, lincosamides, and streptogramins (MLS)	0.016	0.025	0.039	0.019
Multidrug	0.203	0.277	0.230	0.248
Quinolone	0.000	0.002	0.003	0.002
Rifamycin	0.001	0.002	0.000	0.001
Sulfonamide	0.004	0.003	0.003	0.002
Tetracycline	0.009	0.009	0.004	0.003
Trimethoprim	0.002	0.000	0.001	0.001
Unclassified	0.021	0.010	0.010	0.016
Vancomycin	0.002	0.001	0.000	0.001

similar concentrations of ARGs despite differences in wastewater-treatment techniques and/or human activities. Our results suggest that MPs exhibit health threats. The ARGs–OAP pipeline compared the resistome of the plastisphere to resistomes of other environments. The results suggest that wastewater exhibits the resistome related to MPs (Fig. S1). Overall concentrations of ARGs in effluents from FT and XL are higher than those in other environments, which is consistent with the results of previous work (Lu et al., 2019). One of the possible explanations is that wastewater-associated MPs favor vertical gene transfer and horizontal gene transfer (Guo et al., 2018). However, some researchers believe that retention time and the level of antibiotic pollution play significant roles in obtaining ARGs (Lu et al., 2019; Wu et al., 2019). Nonetheless, our results confirm the threats exhibited by the plastisphere and their ecological importance. We believe that further research is needed to understand this new niche of the resistome.

3.3 The degradation of plastics by the plastisphere

In Fig. 1, we can see that the *alkB* gene is densely present in the groups grouped on the basis of water sources and plastic materials (see the Supplementary file for sample information). The density of the *alkB* gene in XL and PET groups is statistically higher than that of the 16S rRNA gene in the groups. The higher production of functional gene copies under extreme conditions has been reported elsewhere (Roux et al., 2011; Long et al., 2021). The families of the *alkB* gene are directly involved in PE degradation. Therefore, the results of our qPCR analysis indicate that both HDPE and PET can be utilized by microbes and serve as the energy sources for the plastisphere. Moreover, metagenomic analyses (e.g., KEGG Mapper and ORFs) indicated chemical reactions related to degradation of plastics (Fig. S2), which implies the degradation of

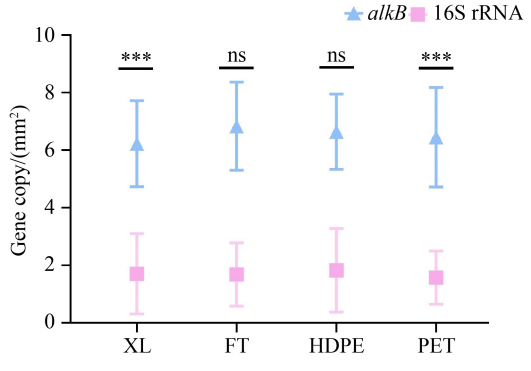


Fig. 1 qPCR measurements of the densities of the *alkB* and 16S rRNA genes. The x-axis shows the groups grouped on the basis plastic materials and water sources, and the y-axis shows the densities (gene copies/mm^2) of the genes. The results of the heteroscedastic student's *t* test (two-tailed) are annotated. *** represents $p < 0.001$, and ns represents "not significant".

HDPE and PET by the plastisphere. The identification of pathways related to chlorinated paraffins (CPs) in our metagenomic datasets indicates that microbes may consume CPs in effluents from WWTPs (Zeng et al., 2012; Zeng et al., 2013) and cause serious environmental concerns (Yuan et al., 2021).

We identified 64 metagenomic contigs that are potentially involved in the activities of PETase and MHETase (Figs. S2(a) and S3), which suggests that the metagenomic samples can utilize PET and its derivatives or byproducts. Further, we made a maximum-likelihood phylogenetic tree to demonstrate the conservation of key MHETase (Fig. 2). The phylogenetic tree showed a high affiliation of the MHETase identified in the metagenome to that of *Comamonadaceae*. *Comamonadaceae* are capable of degrading PET. Yoshida et al. (2016) isolated and discovered a PET-consuming bacterium named *Ideonella sakaiensis* (*I. sakaiensis*). The authors proved that *I. sakaiensis* uses PET as its energy and carbon sources and reported that extracellular PETase hydrolyzes PET to MHET, and MHETase breaks down the MHET. On the basis of bioinformatics analysis, the authors suggested that the metabolisms of PETase and MHETase analogs are established much earlier than their ancestral proteins. Our data showed that not only *I. sakaiensis*, which belongs to the class Betaproteobacteria, but also Alphaproteobacteria and Gammaproteobacteria encoded PETase and MHETase homologs (Figs. 2 and S3). Collectively, these results indicated a broader phylogenetic spectrum of MHET-degrading microorganisms, and we speculate that the MHETase genes can be laterally transferred during wastewater treatment.

3.4 Metagenomics facilitates the understanding of the plastisphere

Recently, Bhagwat et al. (2021) used whole-genome sequencing and explored the microbial structures and functions of lake-associated MPs. Sixteen metagenomic samples were surveyed to study the composition and functional traits of the microbiomes related to MPs. In general, their metagenomic reads were assigned to a set of featured databases (e.g., 16S rRNA and KEGG) for classification and functional inferring. Approximately 15% of the metagenomic reads were analyzed using the KEGG-based Bray–Curtis analysis to describe substrate dissimilarities. Moreover, the relative abundance of selected UniRef genes indicates that the plastisphere exhibits metabolic potential. Likewise, our metagenomic contigs hit map01120 and map00625 pathways ("microbial metabolisms in environments"), and the metabolisms of nitrogen, carbon, and sulfur are the main metabolic activities of the plastisphere (Figs. S4–S6). Additionally, METABOLIC software annotated some other essential metabolic functions, including C1 metabolism, fermentation, methane metabolism, oxygen metabolism, and urea

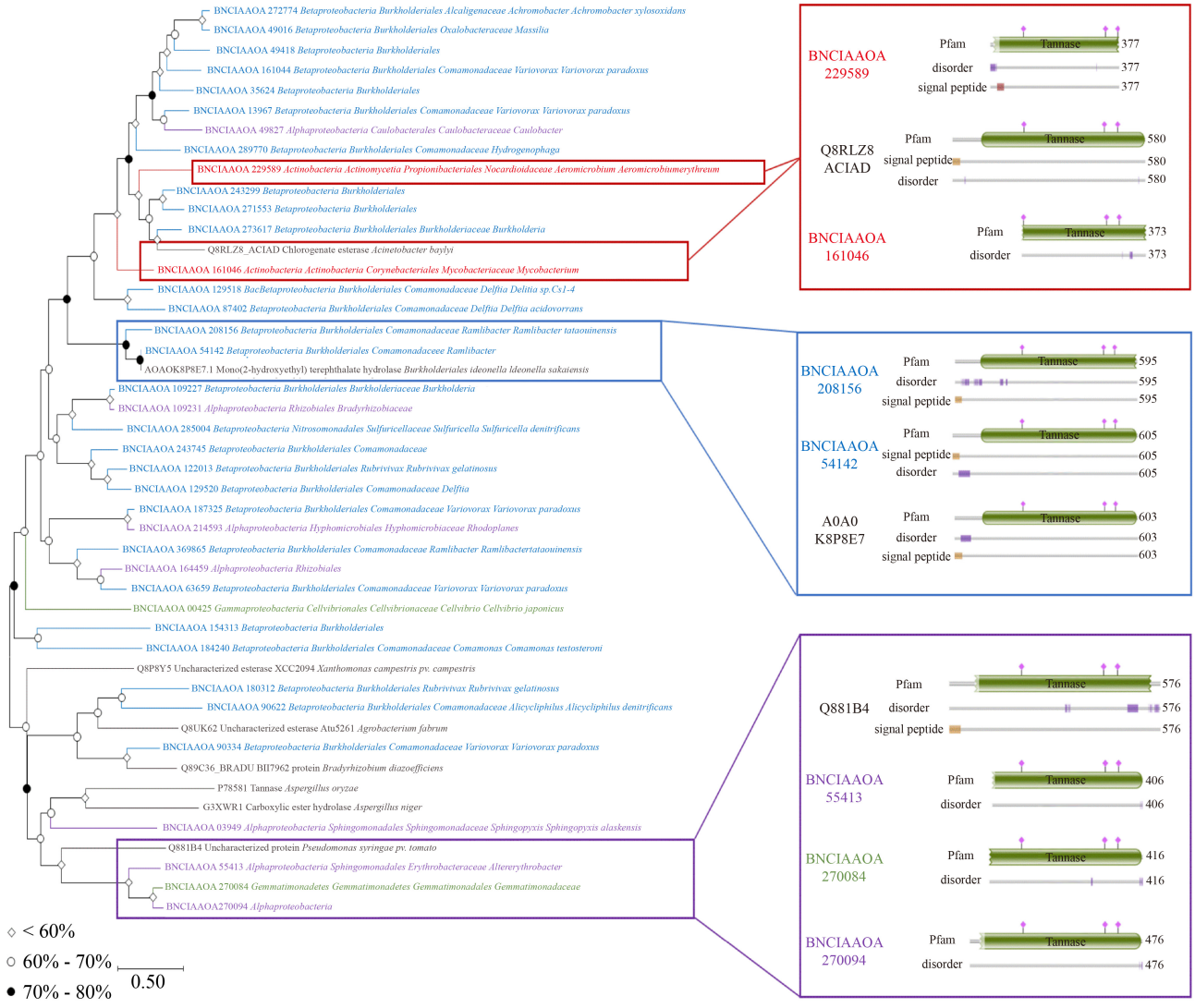


Fig. 2 A maximum-likelihood phylogenetic tree of the bacterial MHETase identified in our metagenome and reference protein sequences.

utilization (the Supplementary file and Fig. S7). The metabolisms of carbon, nitrogen, oxygen, and sulfur correspond to the functionality of Gammaproteobacteria.

One of the advantages of metagenomics is being able to bin and profile microbial genomes. In this study, we successfully obtained 18 MAGs that belonged to the phyla Proteobacteria, Bdellovibrionota, Bacteroidota, Actinobacteria, and Patescibacteria. In MetaWRAP, the "Quant_bin" function calculates the average coverage of a bin (abbreviated as bac) by incorporating the average coverage of the sequencing read of the bin, which indicates the abundance of a genome. In Table S1, we can see that the bac values of FT complement those of XL, which suggests that water sources can shape the community of the plastisphere. Interestingly, in the groups grouped on the basis of plastic materials, most of the MAGs exhibited similar bac values, which suggests that both HDPE and PET may accumulate similar biofilm-forming bacteria at the early stage (i.e., between a few hours and 6 d). MAG5, MAG12, and MAG14 were associated with HDPE, and MAG6 and MAG7 were closely associated with PET. As the biofilms can consume nutrients and plastic chemicals in the effluents, we need further evidence to relate those MAGs to degradation of plastics by conducting chemical analysis, transcriptomics, and metabolomics in combination with SIP. The attachment of bacterial cells to surfaces of plastics depends on the plastics. The addition of hydrophilic functional groups can promote the accessibility of the extracellular enzymes (e.g., exopolysaccharides, depolymerases, and hydrolases) that are critical to initiate the degradation of plastics (Nauendorf et al., 2016). Therefore, the first stage of the formation of the plastisphere is determined by the secretion of extracellular enzymes by microbiomes in effluents. In summary, studying the profile of the early-stage plastisphere is very useful to confirm the presence

of potential plastic degraders.

3.5 Plastic materials and water sources actively select and shape the plastisphere

The orders of the 60 amplicon-sequencing samples are shown in Fig. S8, in which Sphingomonadales, Rhodocyclales, Bdellovibrionales, Rhizobiales, BD7-3, Rhodobacterales, and Burkholderiales are abundant in all of the samples. However, in the samples (e.g., "FT_PET", "XL_HDPE", and "XL_PET"), the relative abundance of the microbial orders on day 14 is different from the relative abundance of the orders on the other time points (Figs. 3(a), 3(b), and 4). The changing patterns suggest that plastic materials may actively select the abundance of microbes during the development of the plastisphere. After incuba-

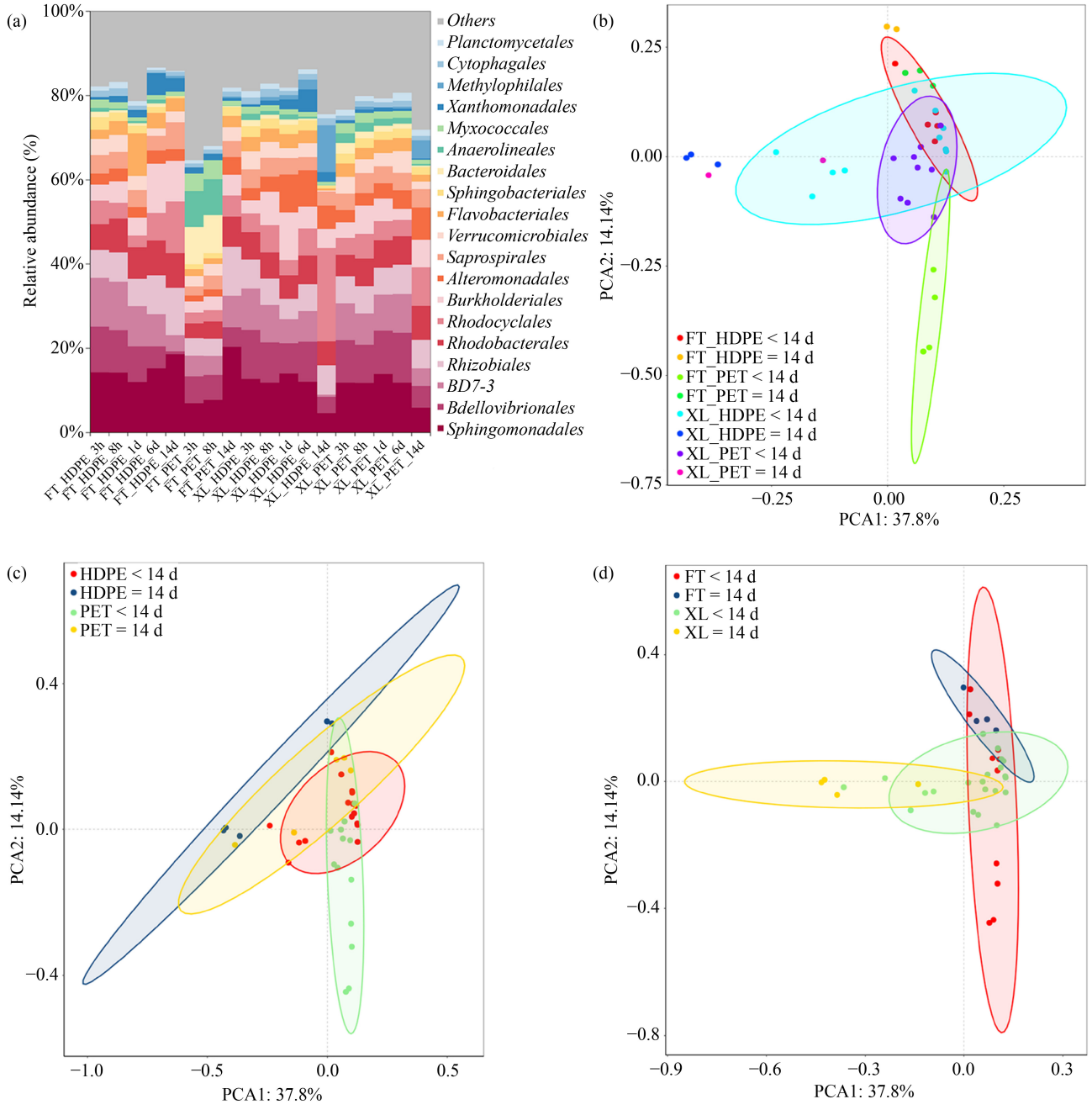


Fig. 3 The clustering of the operational taxonomic units (OTUs) of the plastisphere on the basis of the relative abundance of amplicon 16S rRNA samples. The temporal average relative abundance (ARA) of orders (ARA > 1%) is shown in (a). The principal component analysis (PCA) of the groups grouped on the basis of the combination of plastic materials, water sources, and incubation periods is shown in (b). The PCA of the groups grouped on the basis of the combination of plastic materials and incubation periods is shown in (c), and the PCA of the groups grouped on the basis of water sources and incubation periods is shown in (d).

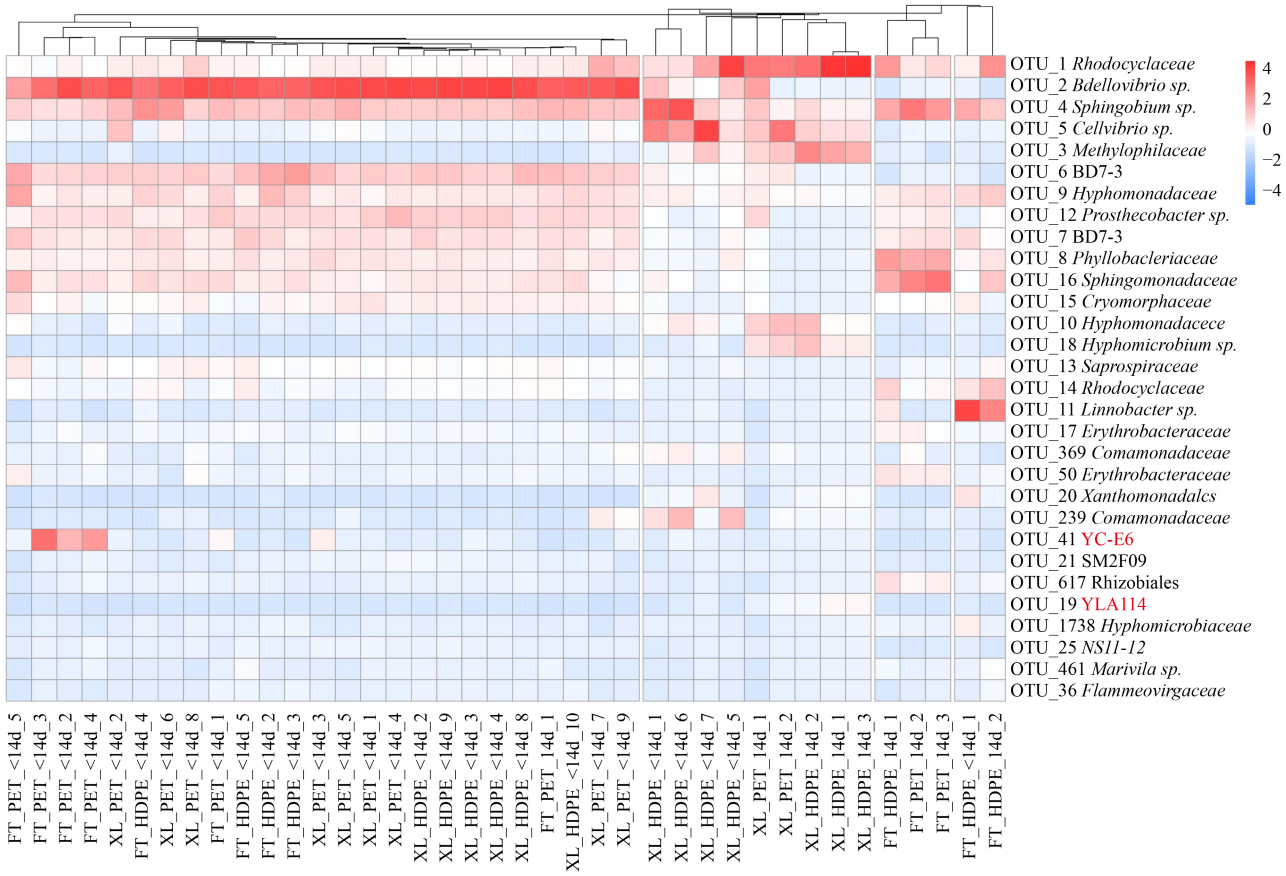


Fig. 4 A heatmap of the operational taxonomic units (OTUs) of the samples of microcosmic experiments. Only the OTU number that is greater than 800 is shown, and the value shows the sample replicates in the *x*-axis. YC-E6 and YLA114 belong to the class Methanomicrobia. Methanomicrobia are indicated in the y-axis by the red color.

tion for 14 d, *Rhodocyclaceae* (OTU_1), *Sphingobium sp.* (OTU_4), *Hyphomonadaceae* (OTU_9), *Phyllobacteriaceae* (OTU_8), and *Sphingomonadaceae* (OTU_16) were dominant in the effluent from FT (Fig. 4). Additionally, *Rhodocyclaceae* (OTU_14) and *Limnobacter sp.* (OTU_11) seemed to actively prefer HDPE to PET. On the contrary, BD7-3 (OTU_6 and OTU_7), *Hyphomonadaceae* (OTU_9), *Prosthecobacter sp.* (OTU_12), and *Cryomorphaceae* (OTU_15) disappeared from the effluent from XL (Fig. 4). Therefore, our heatmap suggests that some microbes gradually become dominant in the plastisphere (i.e., after 14 d of growth). Furthermore, PCA indicated the effect of exposure time on the plastisphere. In the groups grouped on the basis of plastic materials (Fig. 3(c)) or water sources (Fig. 3(d)), the clusters of the biofilms collected on day 14 were distinctly different from those collected before day 14 (between 3 h and 6 d). For instance, after incubation for 14 d, the microcosm of HDPE was distinctly different (Fig. 3(c), ANOSIM, $p < 0.0001$). However, ANOSIM results showed no significant differences among groups grouped on the basis of plastic materials and water sources (Fig. 3(b)), which suggests that the groups share a similar microbial structure at the beginning of the biofilm formation (Fig. 3(a)). This phenomenon can be interpreted on the basis of the fact that the formation of the biofilm is affected by surrounding environments and nutrients (Toyofuku et al., 2016). The results of amplicon sequencing showed that although *Comamonadaceae* (the class Betaproteobacteria) were relatively rare in all amplicon-sequencing samples, they exhibited PET-degrading ability (the Supplementary file). The plastic-degrading species may be rarely present at the beginning, but they play essential roles in the degradation of plastics (rare microorganisms are extensively expressed at the RNA level).

4 Conclusions

Recently, the metagenomic method has been used to explore the functionality of the plastisphere. In the current research, 16S amplicon sequencing and metagenomic sequencing were used to reveal the composition and functionality of the plastisphere, which were influenced by plastic materials and water sources. The degradation of plastic materials by microbes was studied using metagenomics and qPCR. The key findings of the current research were

▪ The MP-related resistome was present in wastewater, which suggests the potential health threats of wastewater.

▪ The *AlkB* gene was densely present, which indicates that HDPE and PET can be utilized by microbes and serve as energy sources for the plastisphere.

▪ Eighteen MAGs that belonged to the phyla Proteobacteria, Bdellovibrionota, Bacteroidota, Actinobacteria, and Patescibacteria were identified.

▪ Calculated MAG coverage values suggested that water sources shaped the microbial community of the plastisphere. Bioinformatics and statistical analysis suggested that plastic materials and water sources actively selected and shaped the plastisphere.

▪ By using metagenomic analysis, we broadened the phylogenetic spectrum of MHET-degrading microorganisms.

Acknowledgements Our research was funded by the National Key Research and Development Program of China (No. 2021YFA1202500), the National Natural Science Foundation of China (No. 42177357), the Guangdong Basic and Applied Research Foundation (China) (No. 2021A1515012191), and the Shenzhen Science and Technology Innovation Committee (China) (No. JCYJ20210324104412033). We thank the Center for Computational Science and Engineering at the Southern University of Science and Technology (China) (SUSTech) and the core research facilities at SUSTech for providing excellent resources and services. Moreover, we thank Dr. Chuanlun Zhang from the Department of Ocean Science and Engineering at SUSTech for lending QuantStudio qPCR.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11783-022-1580-1 and is accessible for authorized users.

References

Aronesty E (2013). Comparison of sequencing utility programs. The Open Bioinformatics Journal, 7(1875−0362): 1–8

Bhagwat G, Zhu Q, O'Connor W, Subashchandrabose S, Grainge I, Knight R, Palanisami T (2021). Exploring the Composition and functions of plastic microbiome using whole-genome sequencing. Environmental Science & Technology, 55(8): 4899–4913

de Tender C A, Devriese L I, Haegeman A, Maes S, Ruttink T, Dawyndt P (2015). Bacterial community profiling of plastic litter in the belgian part of the North Sea. Environmental Science & Technology, 49(16): 9629–9638

Duan J, Bolan N, Li Y, Ding S, Atugoda T, Vithanage M, Sarkar B, Tsang D C W, Kirkham M B (2021). Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. Water Research, 196: 117011

Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, Amato S (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin, 77(1−2): 177–182

Estahbanati S, Fahrenfeld N L (2016). Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. Chemosphere, 162: 277–284

Guo X P, Yang Y, Lu D P, Niu Z S, Feng J N, Chen Y R, Tou F Y, Garner E, Xu J, Liu M, Hochella M F J Jr (2018). Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze Estuary. Water Research, 129: 277–286

Harrison J P, Schratzberger M, Sapp M, Osborn A M (2014). Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. BMC Microbiology, 14(1): 232

Kang D D, Li F, Kirton E, Thomas A, Egan R, An H, Wang Z (2019). MetaBAT 2: An adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ, 7: e7359

Kazour M, Terki S, Rabhi K, Jemaa S, Khalaf G, Amara R (2019). Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. Marine Pollution Bulletin, 146: 608–618

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner F O (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 41(1): e1

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547–1549

Lagarde F, Olivier O, Zanella M, Daniel P, Hiard S, Caruso A (2016). Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environmental Pollution (Barking, Essex : 1987), 215: 331−339

Lechner A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart M, Glas M, Schludermann E (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environmental Pollution (Barking, Essex : 1987), 188(100): 177−181

Lee C, Kim J, Shin S G, Hwang S (2008). Monitoring bacterial and archaeal community shifts in a mesophilic anaerobic batch reactor treating a high-strength organic wastewater. FEMS Microbiology Ecology, 65(3): 544–554

Li X, Chen L, Mei Q, Dong B, Dai X, Ding G, Zeng E Y (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. Water Research, 142: 75–85

Long A M, Hou S, Ignacio-Espinoza J C, Fuhrman J A (2021). Benchmarking microbial growth rate predictions from metagenomes. ISME Journal, 15(1): 183–195

Lu J, Zhang Y, Wu J, Luo Y (2019). Effects of microplastics on distribution of antibiotic resistance genes in recirculating aquaculture system. Ecotoxicology and Environmental Safety, 184: 109631

McCormick A, Hoellein T J, Mason S A, Schluep J, Kelly J J (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. Environmental Science & Technology, 48(20): 11863–11871

Nauendorf A, Krause S, Bigalke N K, Gorb E V, Gorb S N, Haeckel M, Wahl M, Treude T (2016). Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. Marine Pollution Bulletin, 103(1-2): 168–178

Oberbeckmann S, Osborn A M, Duhaime M B (2016). Microbes on a bottle: substrate, season and geography influence community

composition of microbes colonizing marine plastic debris. PLoS One, 11(8): e0159289

Ogonowski M, Schür C, Jarsén Å, Gorokhova E (2016). The effects of natural and anthropogenic microparticles on individual fitness in daphnia magna. PLoS One, 11(5): e0155063

Pham D N, Clark L, Li M (2021). Microplastics as hubs enriching antibiotic-resistant bacteria and pathogens in municipal activated sludge. Journal of Hazardous Materials Letters, 2: 100014

Pinnell L J, Turner J W (2019). Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. Frontiers in Microbiology, 10: 1252

Pinto M, Polania Zenner P, Langer T M, Harrison J, Simon M, Varela M M, Herndl G J (2020). Putative degraders of low-density polyethylene-derived compounds are ubiquitous members of plastic-associated bacterial communities in the marine environment. Environmental Microbiology, 22(11): 4779–4793

Roux S, Enault F, Bronner G, Debroas D (2011). Comparison of 16S rRNA and protein-coding genes as molecular markers for assessing microbial diversity (Bacteria and Archaea) in ecosystems. FEMS Microbiology Ecology, 78(3): 617–628

Seemann T (2014). Prokka: rapid prokaryotic genome annotation. Bioinformatics (Oxford, England), 30(14): 2068−2069

Son H F, Cho I J, Joo S, Seo H, Sagong H Y, Choi S Y, Lee S Y, Kim K J (2019). Rational protein engineering of thermo-stable PETase from ideonella sakaiensis for highly efficient PET degradation. ACS Catalysis, 9(4): 3519–3526

Toyofuku M, Inaba T, Kiyokawa T, Obana N, Yawata Y, Nomura N (2016). Environmental factors that shape biofilm formation. Bioscience, Biotechnology, and Biochemistry, 80(1): 7−12

Wu X, Pan J, Li M, Li Y, Bartlam M, Wang Y (2019). Selective enrichment of bacterial pathogens by microplastic biofilm. Water Research, 165: 114979

Wu Y, Simmons B A, Singer S W (2016). MaxBin 2.0: An automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics (Oxford, England), 32(4): 605−607

Yin X, Jiang X, Chai B, Li L, Yang Y, Cole J R, Tiedje J M, Zhang T (2018). ARGs-OAP v2.0 with an expanded SARG database and Hidden Markov Models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes. Bioinformatics (Oxford, England), 34(13): 2263−2270

Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). Science, 351(6278): 1196–1199

Yuan B, McLachlan M S, Roos A M, Simon M, Strid A, de Wit C A (2021). Long-chain chlorinated paraffins have reached the arctic. Environmental Science & Technology Letters, 8(9): 753–759

Zeng L, Li H, Wang T, Gao Y, Xiao K, Du Y, Wang Y, Jiang G (2013). Behavior, fate, and mass loading of short chain chlorinated paraffins in an advanced municipal sewage treatment plant. Environmental Science & Technology, 47(2): 732–740

Zeng L, Wang T, Ruan T, Liu Q, Wang Y, Jiang G (2012). Levels and distribution patterns of short chain chlorinated paraffins in sewage sludge of wastewater treatment plants in China. Environmental Pollution, 160(1): 88–94

Zettler E R, Mincer T J, Amaral-Zettler L A (2013). Life in the "plastisphere": Microbial communities on plastic marine debris. Environmental Science & Technology, 47(13): 7137–7146

Zhao Z, Baltar F, Herndl G J (2020). Linking extracellular enzymes to phylogeny indicates a predominantly particle-associated lifestyle of deep-sea prokaryotes. Science Advances, 6(16): eaaz4354

Author Biography

Yu Xia et al. Plastic materials & water sources select and shape wastewater plastispheres over time.