Unique Bacterial Community of the Biofilm on Microplastics in Coastal Water

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Abstract

Being immersed in seawater for a few days, microorganisms will adhere to the surface of different materials and form biofilms. After being immersed in seawater for 1 week, high-throughput sequencing method was used to analyze the bacterial community structure of the biofilms on the surface of microbeads with different materials including steel, SiO_2, and polyvinyl chloride (PVC). Operational taxonomic unit clustering results showed that some differences existed in the bacterial communities attached to the surface of different microbeads. Each microbead made by different material had its unique bacterial community. The heatmap indicated that the dominant genera on the surface of different microbeads were different from each other. Quantitative analysis showed that the relative abundance of dominant genera were different among different types of microbeads. Beta diversity analysis and principal component analysis showed that difference in the bacterial community on surface of steel-bead and PVC-bead was the most significant.

Keywords Microplastic · Biofilm · High-throughput sequencing method · Bacterial community · Microbeads

The pollution of microplastics (< 5 mm) is ubiquitous and persistent in oceans, coastal areas, lakes, rivers and polar water (Lu et al. 2019, 2020; Lusher et al. 2015). The presence of microplastics in the aquatic environment poses a great threat to the entire ecosystem and has drawn considerable attention recently (Auta et al. 2017; Law and Thompson 2014). It has been reported that the microplastic abundances ranged from 8 to 9200 particles per m^3 in offshore pacific waters (Auta et al. 2017), and reached up to 8654 items per m^3 at the sea surface (Hidalgo et al. 2012).

After immersed in water for quite a while, the microplastics will be colonized with microorganisms, and microbial biofilms will form on the surface (Harrison et al. 2014). The formation of biofilms on the surface of microplastics promotes the adsorption of harmful microorganisms and pollutants such as heavy metals, hazardous chemicals and persistent organic pollutants on their surface (Harrison et al. 2011; Mincer et al. 2016; Keswani et al. 2016; Rummel et al. 2017; Lu et al. 2019; Zhang et al., 2020). The biofilm formation and enrichment of harmful microorganisms on the microplastics accelerate the adverse ecological effects (Prokic et al. 2019; Zhang et al. 2020). It was found that microplastics might be the carriers of pathogens (Jiang et al. 2018; Oberbeckmann et al. 2015; Zettler et al. 2013; Zhang et al. 2020). To understand the microplastics pollution in the ecosystem clearly, it is very important to study the microbial community of biofilms on microplastic surface.

This study used high-throughput sequencing to investigate the bacterial community of the biofilms formed on the surface of different microbeads (polyvinyl chloride abbreviated as PVC, SiO_2 and steel). PVC is one kind of microplastics, steel represents artificial steel structure facilities, SiO_2 represents clay and sand suspend in the marine. The final

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aim of this study is to provide useful information on the bacterial community structure of the microplastics.

Materials and Methods

Study area was located at a site (37° 27′ 21.85″ N, 121° 42′ 5.99″ E) of Yantai, Shandong Province. The low-carbon steel microbeads (Q235B, $\Phi = 4$ mm), PVC microbeads ($\Phi = 4$ mm), SiO_2 panels ($\Phi = 4$ mm) were put into nylon bags and were immersed in seawater with the depth of 0–0.5 m in September 2017. Nine replicates were used for sampling. The microbeads were collected successively in 0–7 days. The microbeads were scraped by sterilizing stick and washed with sterile fresh seawater to collect the biofilms samples, and then followed by serial centrifugations to pellet for subsequent analysis.

The samples were transported on dry-ice to Majorbio (Shanghai, China) for DNA extraction and sequencing. The total genomic DNA was extracted by CTAB/SDS method. The DNA concentration was determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, USA), and DNA purity was checked by 1% agarose gels. The 16S rRNA genes of regions of V4–V5 were amplified using the universal primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The PCR reaction system (20 μl) contained 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase and 10 ng of template DNA. The PCR amplification was performed using the following procedure: 2 min at 95 °C, followed by 30 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s) and a final extension at 72 °C for 10 min, then held at 4 °C. The PCR products were checked on 2% agarose gel. The bright main strips between 400 and 450 bp were chosen for further extraction. The target PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using QuantiFluor ™-ST (Promega, USA) according to the manufacturer's protocol.

Purified amplicons were analyzed and sequenced on an Illumina HiSeq platform (Illumina, USA) according to the standard protocols by Majorbio Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive database (Accession Number: SRP238994).

Raw reads were assigned, quality-filtered by Trimmomatic, and then merged by FLASH. The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. Primers were exactly matched allowing 2 nucleotides mismatching, and reads containing ambiguous bases were removed. Sequences with overlap longer than 10 bp were merged according to their overlap sequence.

Sequences with similarity over 97% were assigned to the same operational taxonomic units (OTUs) using Uparse (version 7.1). Representative sequence for each OTU was screened using UCHIME for further annotation. The taxonomic information of gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) based on the Silva (SSU123) 16S rRNA database by threshold of 70%. The Shannon index and goods-coverage were calculated by the number of the OTUs and the total sequences detected. The beta diversity shown in the heatmap was calculated by Weighted Unifrac and Unweighted Unifrac values.

Results and Discussions

A total of 205,737 reads with an average length of 372 bp were obtained from the samples. These sequences clustered into 1513 OTUs at a 97% similarity level. The Good's coverages of all the samples were greater than 0.99, suggesting that the sequencing depth of all samples were sufficient to represent bacterial community (Table 1). Shannon diversity estimators varied from 7.337 to 8.237 (Table 1). OTU number and Shannon estimator (Table 1) demonstrated that both parameters were higher in samples Steel_7d and SiO_2_7d than in PVC_7d, indicating that the bacterial community diversities on the surface of the microplastic were relatively less than other microbeads.

The sequences of the samples in this study were classified, and 1513 OTUs were identified in these samples. The Venn diagram (Fig. 1) was used to show the shared and specific OTUs in different types of microbeads. The clustering results showed that some differences existed in the bacterial communities attached to the surface of different microbeads. As shown in Fig. 1, approximately 1306, 1391 and 1496 OTUs were identified on the surface of PVC, SiO_2 and steel microbeads separately. It was indicated that the bacterial community composition on the surface of the steel microbeads are more abundant than those on PVC and SiO_2. The structure of the adherent bacterial community on the surface varied in response to the physical properties of the substrate (Dang and Lovell 2000; Jones et al. 2007; Rosato et al. 2019; Zhang et al. 2019). The number of OTUs shared by PVC

Table 1 Shannon index, coverage and OTUs number of the samples in this study

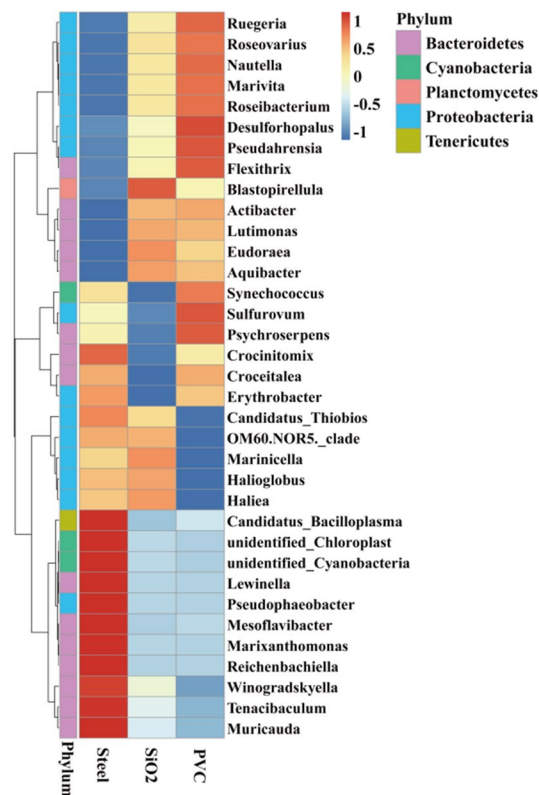| Sample	Shannon[a]	Coverage[b]	OTUs
PVC_7d	7.337	0.997	1423
SiO2_7d	7.646	0.993	1517
Steel_7d	8.237	0.994	1598

[a]The Shannon index is used to calculate the diversity of bacteria community. [b]The coverage is used to evaluate the depth of sequencing
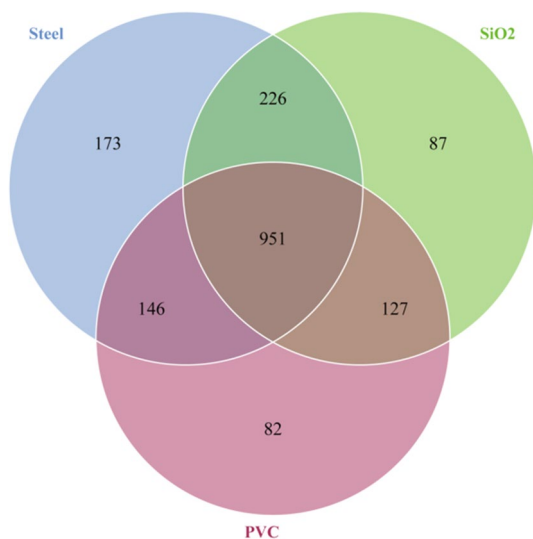
Fig. 1 The shared and specific operational taxonomic units (OTUs) on surface of steel, SiO₂ and polyvinyl chloride (PVC) microbeads by using Venn graph. The digitals in the overlapped parts show the OTUs number shared by different microbeads. The digitals in the unoverlapped parts show the specific OTUs number in different types of microbeads

Fig. 2 The heatmap of the relative abundances of the dominant genera on genus level on the surface of different microbeads, steel, SiO₂ and PVC microbeads

and SiO₂ microbeads was 1078, the number of OTUs shared by PVC and steel microbeads was 1097, and the number of OTUs shared by SiO₂ and steel microbeads was 1177. The number of OTUs shared by PVC, SiO₂ and steel was 951, accounting for more than 60% of the total number of OTUs detected in these samples. It could be concluded that the bacterial community on the surface of different types of microbeads included many common genera.

The heatmap in Fig. 2 showed the dominant genera on genus level on the surface of different microbeads. The dominant genera on the surface of the steel microbeads were *Muricauda*, *Tenacibaculum*, *Winogradskyella*, *Reichenbachiella*, *Marixanthomonas*, *Mesoflavibacter*, *Pseudophawobacter*, *Lewinella*, unidentified_*Cyanobacteria*, unidentified_*Chloroplast*, Candidatus_*Bacilloplasma* and *Crocinitomix*. The dominant genera on the surface of the SiO₂ microbeads were *Blastopirellula*, *Actibacter*, *Lutimonas*, *Eudoraea*, *Aquibacter*, *Haliea*, *Halioglobus* and *Marinicella*. The dominant genera on the surface of the PVC microbeads were *Ruegeria*, *Roseovarius*, *Nautella*, *Marivita*, *Roseibacterium*, *Desulforhopalus*, *Pseudahrensia*, *Flexithrix*, *Sulfurovum*, *Psychroserpens* and *Synechococcus*. The heatmap in Fig. 2 indicated that the dominant genera on the surface of different microbeads were different from each other. *Haliea*, *Halioglobus*, *Marinicella*, OM60. NOR5._clade and Candidatus_*Thiobios* were the 5 genera with higher abundance detected on the surface of steel and SiO₂ microbeads. *Synechococcus*, *Sulfurovum*, *Psychroserpens*, *Crocinitomix*, *Croceitalea* and *Erythrobacter* were the

6 genera with higher abundance detected on the surface of steel and PVC microbeads. *Ruegeria*, *Roseovarius*, *Nautella*, *Marivita*, *Roseibacterium*, *Desulforhopalus*, *Pseudahrensia*, *Flexithrix*, *Blastopirellula*, *Actibacter*, *Lutimonas*, *Eudoraea* and *Aquibacter* were the 13 genera with higher abundance detected on the surface of SiO₂ and PVC microbeads. The dominant bacteria on the surface of SiO₂ and PVC microbeads showed higher similarity while lower similarity was found between SiO₂ and steel microbeads or PVC and steel microbeads. The bacterial community structure on the surface of steel microbeads was quite different from that of SiO₂ and PVC microbeads. It might be caused by that the surface of steel was prone to corrode after being immersed into water to subsequently affect the microbial community structure on the surface (Dang and Lovell 2015).

The bar chart in Fig. 3 showed the quantitative results of the relative abundance of 28 dominant genera (over than 0.5%) on surfaces of several types of microbeads. The total relative abundance of the 28 dominant genera was less than 50%, indicating that the bacterial community in these samples was relatively complex. As shown in Fig. 3, the dominant genera (with the relative abundance over than 2%) on the surfaces of steel microbeads were *Marivita*, unidentified_*Chloroplast*, *Ruegeria*, *Marinicella*, *Actibacter*,
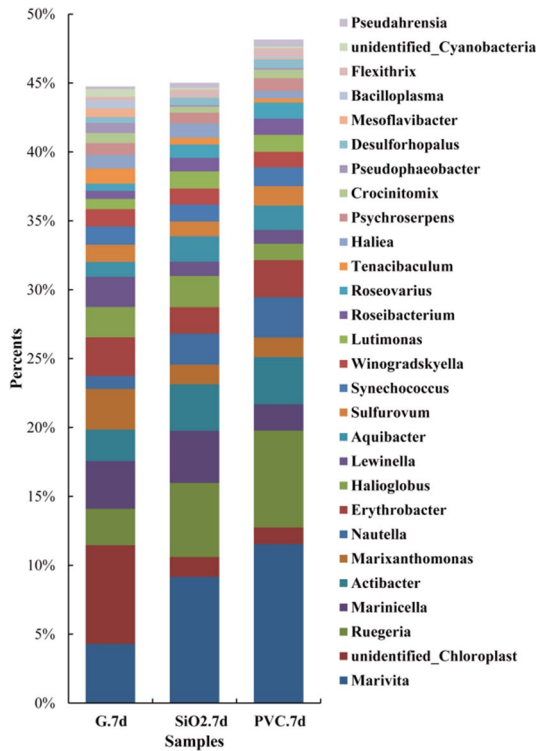
Fig. 3 The bar chart of the relative abundance of 28 dominant bacteria (over than 0.5%) on surfaces of types of microbeads on genus level. The relative abundance is the percentage of the bacteria in the total bacterial detected

Marixanthomonas, *Erythrobacter*, *Halioglobus* and *Lewinella*. The dominant genera (with the relative abundance over than 2%) on the surfaces of SiO_2 microbeads were *Marivita*, unidentified_*Chloroplast*, *Ruegeria*, *Marinicella*,

Actibacter, *Nautella* and *Halioglobus*. The dominant genera (with the relative abundance over than 2%) on the surfaces of PVC microbeads were *Marivita*, *Ruegeria*, *Actibacter*, *Nautella* and *Erythrobacter*, and the relative abundance of these genera was higher than steel and SiO_2 microbeads, indicating that these 5 genera were the characteristic attached bacteria of microplastic particles. It was previously found that the dominant bacteria on the surface of microplastics collected from the marine were *Muricauda*, *Ruegeria*, *Psychroserpens*, *Planctomyces*, *Roseovarius* and *Mycobacterium* (Lu et al. 2019). The genera *Ruegeria*, *Psychroserpens* and *Roseovarius* were found with higher abundance (more than 0.5%) on the surface of PVC microbeads in this study.

As shown in Fig. 3, the relative abundance of three genera including unidentified_*Chloroplast*, *Marixanthomonas* and *Lewinella* on the surface of steel microbeads was higher than that of SiO_2 and PVC microbeads. The relative abundance of bacteria genera including *Marivita*, *Ruegeria*, *Actibacter*, *Nautella* and *Aquibacter* on the surfaces of SiO_2 and PVC microbeads was higher than that of steel microbeads. The relative abundance of *Marinicella* and *Halioglobus* on the surfaces of steel and SiO_2 microbeads was higher than that of PVC microbeads while the abundance of *Erythrobacter* on the surface of SiO_2 microbeads was far lower than that of steel and PVC microbeads. These results suggested that the relative abundance of dominant genera were different among different types of microbeads. The results were consistent with previous studies on the microbial communities of different substrates (Zhang et al. 2019; Rosato et al. 2019).

The beta diversity of the bacterial community on different types of microbeads was shown in Fig. 4a. The beta diversity index between samples was less than 0.3, indicating that there was no significant difference in the bacterial
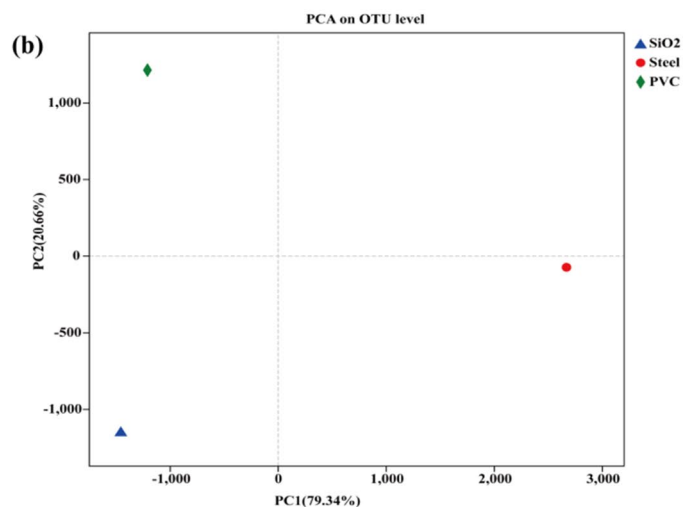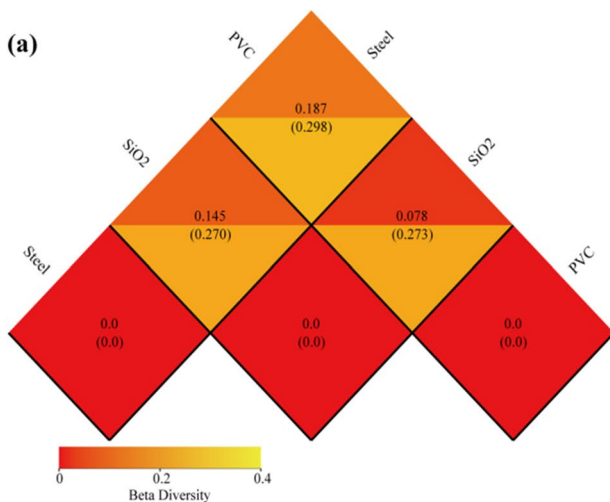
Fig. 4 Beta diversity analysis and principal component analysis of the bacterial community on different types of microbeads, steel, SiO_2 and PVC microbeads

communities among different microbeads. In addition, beta diversity analysis in Fig. 4a showed that the bacterial community difference between SiO_2 and PVC was the smallest, followed by the difference between steel and SiO_2, and the bacterial community difference between steel and PVC was the greatest. The principal component analysis of the samples on OTU level showed that on the PC1 axis (79.34%), the difference between Steel and SiO_2, PVC samples was larger than the difference between SiO_2 and PVC. The bacterial community on the surface of the steel microbeads was greatly different from that of the other two types of microbeads, which was consistent with that of heatmap analysis. The corrosion of steel might affect the bacterial community on surface of steel microbeads.

Compliance with Ethical Standards

Conflict of interest All the authors declare that they do not have any conflicts of commercial or associative interest to this work.

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