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Microbial biofilm formation and community structure on low-density polyethylene microparticles in lake water microcosms

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ABSTRACT

The occurrence of microplastics (MPs) in the environment has been gaining widespread attention globally. MP-colonizing microorganisms are important links for MPs contamination in various ecosystems, but have not been well understood. To partially address this issue, the present study investigated biofilm formation by microorganisms originating from lake water on low-density polyethylene (LDPE) MPs using a cultivation approach and the surface-related effects on the MP-associated microbial communities using 16S rRNA high-throughput sequencing. With the addition of nonionic surfactants and UV-irradiation pretreatment that changed the surface properties of LDPE MPs, more microorganisms were colonized on LDPE surface. Microbial community analysis indicated that LDPE MPs were primarily colonized by the phyla Proteobacteria, Bacteroidetes and Firmicutes, and the surface roughness and hydrophobicity of MP were important factors shaping the LDPE MP-associated microbial community structure. Half of the top 20 most abundant genera colonizing on LDPE were found to be potential pathogens, e.g., plant pathogens *Agrobacterium*, nosocomial pathogens *Chryseobacterium* and fish pathogens *Flavobacterium*. This study demonstrated rapid bacterial colonization of LDPE MPs in lake water microcosms, the role of MPs as transfer vectors for harmful microorganisms in lake water, and provided a first glimpse into the effect of surface properties on LDPE MP-associated biofilm communities.

1. Introduction

Plastic pollution has been gaining public and regulatory attention globally, particularly pollution problems across estuarine and marine ecosystems (Derraik, 2002; Li et al., 2016; Peng et al., 2017). Microplastics (MPs) are commonly defined as plastic particles with diameters smaller than 5 mm (Thompson et al., 2004), which are largely derived from photochemical and mechanical breakdown of larger plastics (Andrady, 2011; Bouwmeester et al., 2015; Lambert and Wagner, 2016) or industrial pristine resins, e.g., plastic pellets in personal care products (Fendall and Sewell, 2009; Gregory, 1996). MPs are identified as vectors for the accumulation and transport of plastic additives and organic pollutants (Bakir et al., 2016; Ziccardi et al., 2016), as well, recognized to provide sites for microbial colonization and biofilm formation (Harrison et al., 2014). Because microorganisms colonized on MPs provide key ecosystem functions, the studies of microbial-microplastic interactions become an increasing important topic in microplastic pollution.

One of the essential significance to characterize microbial communities on MPs is to explore the potential of MPs functioning as transport vector for pathogenic microorganisms (Kirstein et al., 2016; Masó et al., 2003; Shapiro et al., 2014; Zettler et al., 2013). The presence of pathogens in open water may not be problematic because of dilution by water, but formation of biofilm on plastics would enhance the concentrations of pathogenic microorganisms (Lyons et al., 2010). Plastic particles can be more rapidly transported by drifting than individual bacterial cells, facilitating the spread of pathogens and increasing their toxic potential in aquatic ecosystems. Due to the small size of MPs, they are easily ingested and accumulated by a wide range of animals through the food web, along with the toxic substrates and colonized microorganisms (Oberbeckmann et al., 2015a). Potentially pathogenic Vibrio spp. are frequently detected on marine (micro)plastic (Oberbeckmann et al., 2015a), *and Vibrio parahaemolyticus* has been identified on MP particles of polyethylene, polypropylene and polystyrene in marine environments (Kirstein et al., 2016).

To date, although there is an increasing number of studies, mostly within the context of marine environments, to characterize MP-associated microbial biofilm communities (Oberbeckmann

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et al., 2015a; Harrison et al., 2018), each of them shows a fragment of the overall picture of natural diversity of MP-colonizing communities and potential adverse effects of MPs pollution. Therefore, the analyses of MPs-associated biofilm in all different environmental compartments are now greatly encouraged to enhance our knowledge of this research area. As well, many factors influencing the formation, composition and activities of plastic-colonizing communities have not been fully investigated with reference to MPs, especially in freshwater environments. Using a culture-dependent approach, the present study aimed to investigate the colonization of MPs by microbial assemblages originating from lake water with fishing activities. Two particle size of low-density polyethylene (LDPE) MPs were used as the substratum and vector for lake prokaryotic biota; the addition of nonionic surfactant and the pretreatment of UV-irradiation were employed to modify the surface chemistry and structure of LDPE MPs. Using high-throughput sequencing of 16S rRNA genes, the lake water derived microbial communities inhabiting on LDPE MPs were characterized and the potential pathogens were identified. Our results demonstrated the function of LDPE MPs as microscopic island for microbial pathogen in lake water, and the importance of surface properties (roughness and hydrophobicity) shaping LDPE MP-associated communities in freshwater habitats.

2. Materials and methods

2.1. Materials

Microparticles of LDPE with sizes 1000 and 5000 μm were purchased from Goodfellow Cambridge (Huntingdon, UK). They were denoted as LDPE1000 and LDPE5000, and were used in the cultivation experiments as supplied. All chemicals used were of analytical grade and were used as received without any treatment.

2.2. Lake water-LDPE MPs microcosms

Lake water was collected from a small lake located on the campus of Jinan University, China (E°113.36, N°23.14). To harvest enough biofilm biomass for further analyses, the lake water containing 0.5 g L^{-1} yeast extract, 1.0 g L^{-1} peptone and 1.0 g L^{-1} NaCl was used for the culturing experiments. In some microcosms, one of the nonionic surfactants (Triton X-100, Tween 20 or Tween 80) was added to the lake water at 0.01%, 0.1% or 1.0% to test the effects of surfactant type and amount on microbial colonization of LDPE MPs. Exposure to UV irradiation can change the surface chemistry and structure of MPs, partial LDPE MPs were subject to artificial UV irradiation (with a UV light at 254 nm and 40 W) for 30 d, which also simulated natural weathering of LDPE MPs exposed to sun lights in the environment. The UV chamber was maintained at room temperature during UV exposure. The eight microcosms are detailed in Table S1 ("S" indicates tables and figures in the Supplementary material afterwards). A lake water-MPs microcosm was conducted in a 500-ml flask containing 200 ml culture media, 5 g L^{-1} MPs, and surfactant (if required) and incubated on a rotary shaker (100 rpm) at 25 °C. MP samples were taken when the growth of suspended cells reached a maximum for the further analysis of biofilm biomass, biofilm viability and biofilm community structures.

2.3. Bradford determination of protein concentrations

As the amount of extractable protein is directly proportional to the number of adhered microorganisms (Tribedi and Sil, 2013), the bacterial biomass in biofilm on LDPE MPs was estimated by determination of protein concentration using the Bradford method (Sedmak and Grossberg, 1977). At the post-exponential-phase of culturing (4 days after set-up), microplastic pieces (0.1 g) were separated from the culture media and washed by vortexing in normal saline (0.9%) for 10 min to remove adherent cells from MPs. An aliquot (20 μL) of the supernatant was mixed with 200 μL of Bradford protein assay reagent (Sangon Biotech, China) and incubated for 5 min at room temperature, and its absorbance was measured at 595 nm with a microplate reader (Synergy H1, BioTek, USA).

2.4. Determination of biofilm viability

The microbial activity of biofilms was determined using Cell Counting Kit 8 (CCK-8). The cells adsorbed on LDPE MPs were first collected using the same protocol for protein content measurement (in section 2.3). The microbial cell solution (190 μL) was then inoculated in a 96 well microplate and mixed with 10 μL of reagent solution, and its absorbance was measured at 450 nm with a microplate reader (Synergy H1, BioTek, USA) after 4-h incubation at 37 °C.

2.5. Fourier transform infrared analysis of LDPE microplastics

The chemical structures of LDPE MPs before and after surfactant addition (at a concentration of 0.10%) or UV preirradiation were characterized with a Thermo Scientific Nicolet iS50 attenuated total reflectance-Fourier transform infrared (FTIR) spectrometer (Waltham, MA, USA) within the frequency range of 400–4000 cm^{-1}. All LDPE MP pieces were washed with Milli-Q water three times, dried in a vacuum desiccator and stored in sterilized centrifuge tubes before FTIR analysis.

2.6. Scanning electron microscope (SEM) imaging

After cultivation, microplastic particles were removed from the media and immediately fixed in 2.5% glutaraldehyde in PBS medium for 5 h at room temperature. After fixation, the samples were washed six times with PBS medium (pH = 7) and dehydrated with a series of ethanol solutions (30%, 50%, 70%, 90% and 95% each for 10–15 min and three times with 100% ethanol for 15 min). They were allowed to stay overnight and dried in a vacuum desiccator for 12 h (Bo et al., 1983). The samples were gold-coated and visualized with a Zeiss Ultra 55 field-emission scanning electron microscope (Germany), operated at 10 kV.

2.7. High-throughput sequencing of the V4 region of 16S rRNA genes

MP-associated biofilm was first removed from 1.0 g of LDPE MPs using the same method described in section 2.3. Total genomic DNA was then extracted with the Bacteria Genomic DNA Kit (Zomanbio, Beijing, CHN) following the manufacturer's protocol. Total genomic DNA was subject to 16S rRNA gene amplification and 16S rRNA gene sequencing using an Illumina MiSeq platform at Ecogene Biotech (Shenzhen, China). Briefly, the 16S rRNA amplicons were amplified using primer pair 515F/806R (515F: 5′-GTGYCAGCMGCCGCGGTAA-3′, 806R: 5′- GGACTACHVGGGTWTCTAAT-3′), targeting the V4 hyper variable regions of 16S rRNA genes. Each 30 μL PCR mix included 0.75 units Ex Taq DNA polymerase (TaKaRa, Dalian, China), 1 × Ex Taq buffer (TaKaRa, Dalian, China), 0.2 mM dNTP mix (TaKaRa, Dalian, China), 0.2 μM of each primer and 100 ng template DNA. The PCR amplification program consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 53 °C for 60 s and 72 °C for 60 s, and a final elongation at 72 °C for 7 min.

The raw reads were merged using FLASH Version 1.2.11 (Magoč and Salzberg, 2011) and the merged sequences were subsequently analyzed by QIIME version 2018.6 (Caporaso et al., 2010). Demultiplexing was summarized using demux (https://github.com/qiime2/q2-demux) and plugins q2-dada2 (https://github.com/qiime2/q2-dada2) (Callahan et al., 2016) was used for quality control, chimeric sequences removal and sequences clustering. Taxonomic analyses were conducted by using plugins classifier-sklearn (https://github.com/qiime2/q2-feature-classifier) and the SILVA version 132 database (Quast et al., 2013).

The operational taxonomic unit (OTU) table was rarefied to the minimum sample count (68,849 sequences) for subsequent analysis including α- and β-diversity. α-diversity measurements including number of observed OTUs (based on 100% sequence similarity), ACE, Chao1 estimator, Shannon Index and Simpson Index were calculated to characterize microbial composition. β-diversity metrics were applied using the QIIME2 (https://qiime2.org) command line interface (v.2018.6). The similarity among the microbial communities was determined by principal coordinate analysis (PCoA) based on weighted UniFrac distance.

2.8. Statistical analyses

All experiments were performed in triplicates, and data are presented as the mean ± standard deviation. The significant differences among treatments were performed by one-way ANOVA using the SPSS software. A P value < 0.05 was set as the significant level for all analyses.

3. Results and discussion

3.1. Effects of nonionic surfactants on microbial colonization of LDPE microplastics

The addition of surfactants into the culture medium increased the protein concentrations of biomass on both LDPE1000 and LDPE5000 (Fig. 1). Tween 80 was shown to be most effective in facilitating bacteria colonization of LDPE MPs. For LDPE1000, the addition of 0.01%, 0.1% and 1% Tween 80 increased the protein concentration of biomass from 501 ± 29.8 to 685 ± 14.9, 1066 ± 81.9 and 829 ± 29.8 μg g^{-1} MPs, respectively. For LDPE5000, the amendment of 0.01%, 0.1% and 1% Tween 80 enhanced the protein concentration of biomass from 827 ± 14.9 to 1143 ± 14.9, 1824 ± 67.0 and 1672 ± 44.6 μg g^{-1} MPs, respectively. Regardless of the type of surfactants, the concentration of 0.1% (among 0.01%, 0.1% and 1%) seemed to be most suitable for enhancing biofilm formation. SEM photomicrographs (Fig. 2a) also indicated that Tween 80 and Tween 20 at a concentration of 0.1% enhanced bacterial colonization on LDPE MPs, especially LDPE5000; while the effect of Triton X-100 amendment on biofilm formation was not visually detected in SEM (data not shown). Therefore, for further analysis of microbial community structure of MP-associated biofilm, 0.1% of Tween 80 was employed to enhance biofilm formation on LDPE MPs.

Same as many other synthetic polymers, the hydrophobic surfaces of LDPE MPs are unfavorable for close contact between microorganism and MPs, i.e., microbial colonization and biofilm formation become slow on MP surfaces (Karlsson et al., 1988). Nonionic surfactants are amphipathic molecules with both hydrophobic and hydrophilic moieties, one of which is adsorbed on the surface depending on the nature of the surface. In the case of LDPE, surfactants are adsorbed with hydrophobic groups oriented towards the surface, becoming more hydrophilic (Rosen and Kunjappu, 2012). FTIR spectra of LDPE MPs in the presence and absence of surfactants are displayed in Fig. S1. Without surfactants, strong peaks of the FTIR spectra of LDPE1000 and LDPE5000 occurred in the regions of 1440–1490 and 700-750 cm^{-1}, corresponding to the vibrations of wagging and rocking methylene (CH_2) that are characteristic of infrared spectroscopic patterns of LDPE (Albertsson et al., 1987). The hydrophilic moiety in Triton X-100, Tween 80 and Tween 20 is ethylene oxide. The characteristic adsorption band of C-O-C is a wide strong peak at 1110 cm^{-1}, which was observed in the spectra of LDPE MPs with the addition of surfactants. Clearly, the presence of surfactants decreased the hydrophobicity of LDPE surfaces they were adsorbed on, facilitating the contact between microorganisms and LDPE MPs.

3.2. Effects of UV pre-irradiation on microbial colonization of LDPE microplastics

Regardless the presence and the type of surfactant, the protein concentrations of biofilms formed on UV-pretreated LDPE MPs were higher than those on untreated LDPE MPs (Fig. 3a). For example, the protein amounts of biomass on UV-pretreated LDPE1000 and LDPE5000 were determined to be 917 ± 88.6 and 1504 ± 56.6 μg g^{-1} LDPE, which was 84% and 68% higher than the untreated LDPE1000 and LDPE5000, respectively. The viability of biofilm on UV-pretreated LDPE MPs was either comparable with ($p > 0.05$) or higher than ($p < 0.05$) that of untreated LDPE MPs (Fig. 3b). Shown in SEM, the surface of UV-pretreated LDPE MPs contained a larger number of bacteria (Fig. 2b) than that of untreated LDPE MPs, especially for LDPE5000 MPs. UV pretreatment caused an evident morphological change of LDPE5000 surface (Fig. S2), i.e., the surface became rougher and had more pores, cracks or grooves compared with the smooth surface of untreated LDPE5000. The surface change of LDPE1000 upon UV-irradiation was also visible, although the extent was less than LDPE5000 (Fig. S2).

Photo-oxidation generally causes degradation of polymers, resulting in the breakage of polymer chains and the formation of carbonyl (C=O) and vinyl ($CH=CH_2$) groups (Ojeda et al., 2011). The hydrophicility of polymers generally would increase by the introduction of hydrophilic groups of carbonyl (Albertsson et al., 1987). The infrared absorption bands in the ranges of 1600–1800 cm^{-1} and 850-1300 cm^{-1} correspond to stretching vibration of the carbonyl functional group (C=O) and wagging and rocking vibrations of vinyl groups, respectively. In the present study, the FTIR spectra (Fig. S3) implicated the potential formation of carbonyl and vinyl groups in both UV-pretreated LDPE1000 and LDPE5000. Thus, the UV-pretreated LDPE MPs with higher hydrophicility were more susceptible to microbial attack than the untreated LDPE MPs. Since contact angle is linearly correlated to hydrophobicity, it is generally used as an indicator of polymer surface hydrophobicity. Here the contact angle of LDPE5000 and LDPE5000/UV was measured to be 122° and 98°, respectively (Fig. S4). Accordingly, UV-pretreated LDPE5000 was less hydrophobic than untreated LDPE5000, favoring microbial adhesion and biofilm formation.

3.3. Structure and taxonomic composition of biofilm on LDPE particles

Microbial community analysis demonstrated that all LDPE MPs were colonized by biofilms containing diverse microbial assemblages. A total of 8400 observed OTUs were generated from 24 samples, with an average of 350 OTUs per sample. LDPE MP-associated biofilms were predominant by the phyla Proteobacteria, Bacteroidetes and Firmicutes (Fig. S5), which are common components of freshwater biofilm (Rickard et al., 2003; Besemer et al., 2007; Hoellein et al., 2014). Proteobacteria and Bacteroidetes are often observed as the early biofilm colonizers of plastic

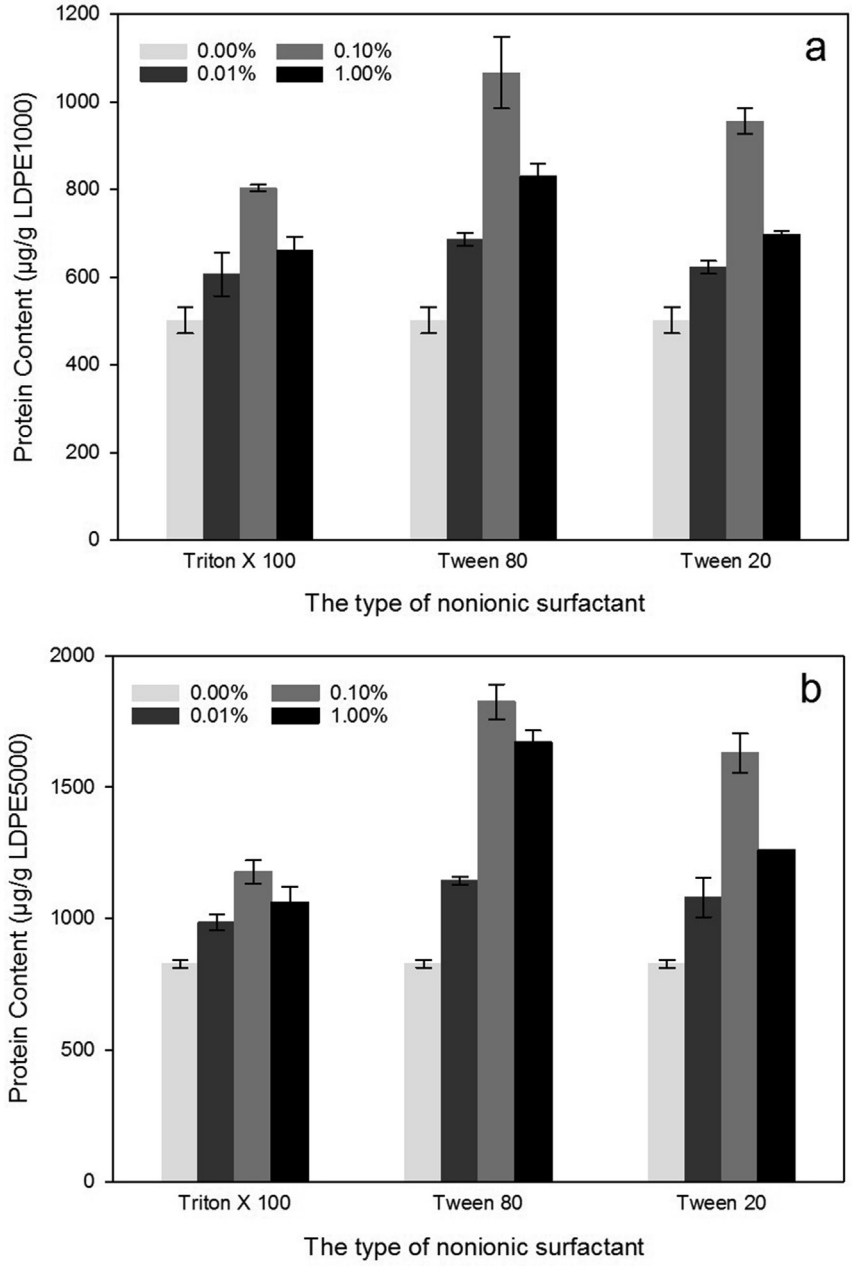

Fig. 1. The amount of protein in the biofilm on LDPE MPs sampled from microcosm experiments with and without the amendment of nonionic surfactant (Triton X100, Tween 80 or Tween 20 at concentrations of 0.01%, 0.10% and 1.0%). a) LDPE MPs with particle size of 1000 μm, b) LDPE MPs with particle size of 5000 μm.

in both marine (De Tender et al., 2015; Oberbeckmann et al., 2015a) and river environments (Jiang et al., 2018; McCormick et al., 2014). This suggested that biofilms of early stage were formed on LDPE MPs during the culture period in the present study. Firmicutes is not frequently detected in (micro)plastic microbial communities in marine environments, but its high relative abundance has been found as the most notable feature of the pond biofilm communities (Hoellein et al., 2014). Firmicutes is known to be ubiquitous in soil, and is also predominant among the typical sewage-associated microorganisms (Oberbeckmann et al., 2015b). Therefore, soil or sewage may serve as a possible source of Firmicutes microorganisms in the biofilm on LDPE MPs in the present study. This implicates that MPs are facilitators of sedimentary microorganisms to new environments via rafting. Microorganisms from the phylum Firmicutes are capable of degrading a variety of organic pollutants, such as polychlorinated biphenyl (Gomes et al., 2014), petroleum hydrocarbons (Fuentes et al., 2014), and hexahydro-1,3,5-trinitro-1,3,5-triazine (Cupples, 2013).

At family level, the top 10 families included Enterobacteriaceae, Moraxellaceae, Comamonadaceae, Oxalobacteraceae, Caulobacteraceae, Aeromonadaceae, Pseudomonadaceae, [Exiguobacteraceae], Flavobacteriaceae and Bruellaceae (Fig. 4). Of them, Moraxellaceae, Comamonadaceae, Aeromonadaceae, Pseudomonadaceae and Flavobacteriaceae have been identified as the abundant families in freshwater microplastic-colonizing communities (McCormick et al., 2014; Jiang et al., 2018). It is very evident that UV-pretreatment greatly altered the relative abundances of the widely distributed families within the MP-colonizing biofilm. Compared with untreated LDPE MPs, LDPE5000/UV harbored more Enterobacteriaceae (75.3% of the total bacterial abundance) at the expense of

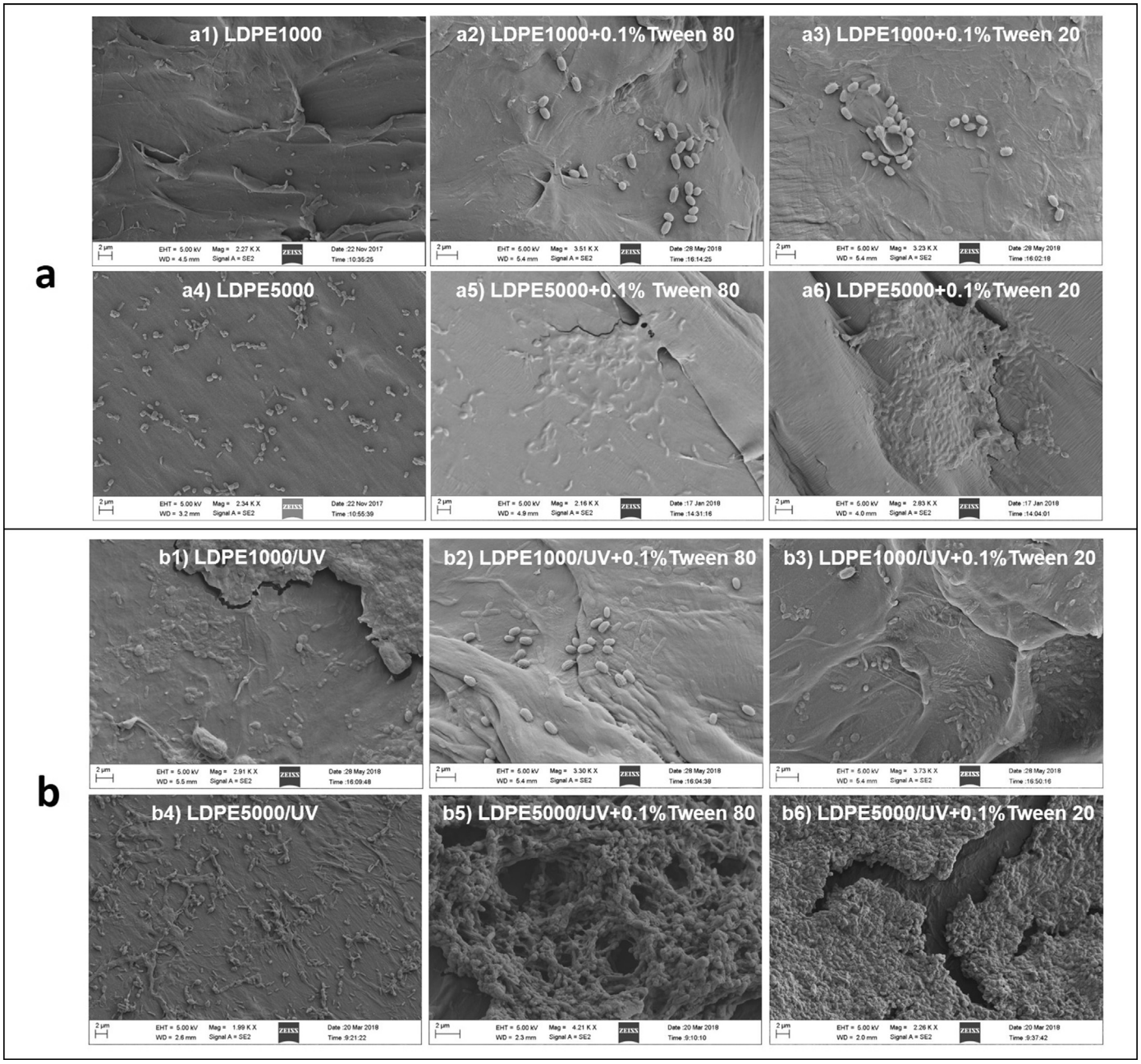

Fig. 2. Scanning electron micrographs showing microbial colonization of LDPE MPs: a1)-a6): with and without the amendment of nonionic surfactant (0.1% Tween 80 or 0.1% Tween 20), b1)-b6): with and without the pretreatment of UV-irradiation.

Comamonadaceae, Flavobacteriaceae and Bruellaceae, while Oxalobacteraceae (52.4%) became the most predominant family in LDPE1000/UV. The presence of surfactant increased the abundance of Moraxellaceae, which is composed by a heterogeneous group of bacteria having diverse clinical significances (Teixeira and Merquior, 2014).

In the present study, *Bacillus* was identified as one of the top 20 most abundant genera (Fig. S6), and LDPE1000 and LDPE1000 + Tw80 contained higher relative abundance of *Bacillus* than other LDPE samples. To date, PE-degrading bacteria are frequently belonging to *Bacillus,* for example, *Bacillus cereus* (Auta et al., 2017; Sowmya et al., 2014), *B. pumilus*, *B. subtilis* (Harshvardhan and Jha, 2013) and *B. gottheilii* (Auta et al., 2017) have been reported to be capable of degrading polyethylene. Besides plastic-degrading bacteria, some fungal taxa are also known to be capable of degrading plastics (Shah et al., 2008). SEM did not show any fungal hyphae on LDPE MPs in the present study, but possible fungal colonization can be further considered using molecular approaches.

In Table 1, the ACE and Chao1 indices are applied for evaluating community richness; while the Shannon and Simpson indices are used for evaluating community diversity. Without UV pre-irradiation and surfactant addition, the difference in community richness and diversity between LDPE1000 and LDPE5000 was insignificant, which was consistent with previous observation that the α-diversity of microplastic community was independent of microplastic size within a range of 0.3–5 mm (Frère et al., 2018). However, for larger plastic, particle size was found to influence α-

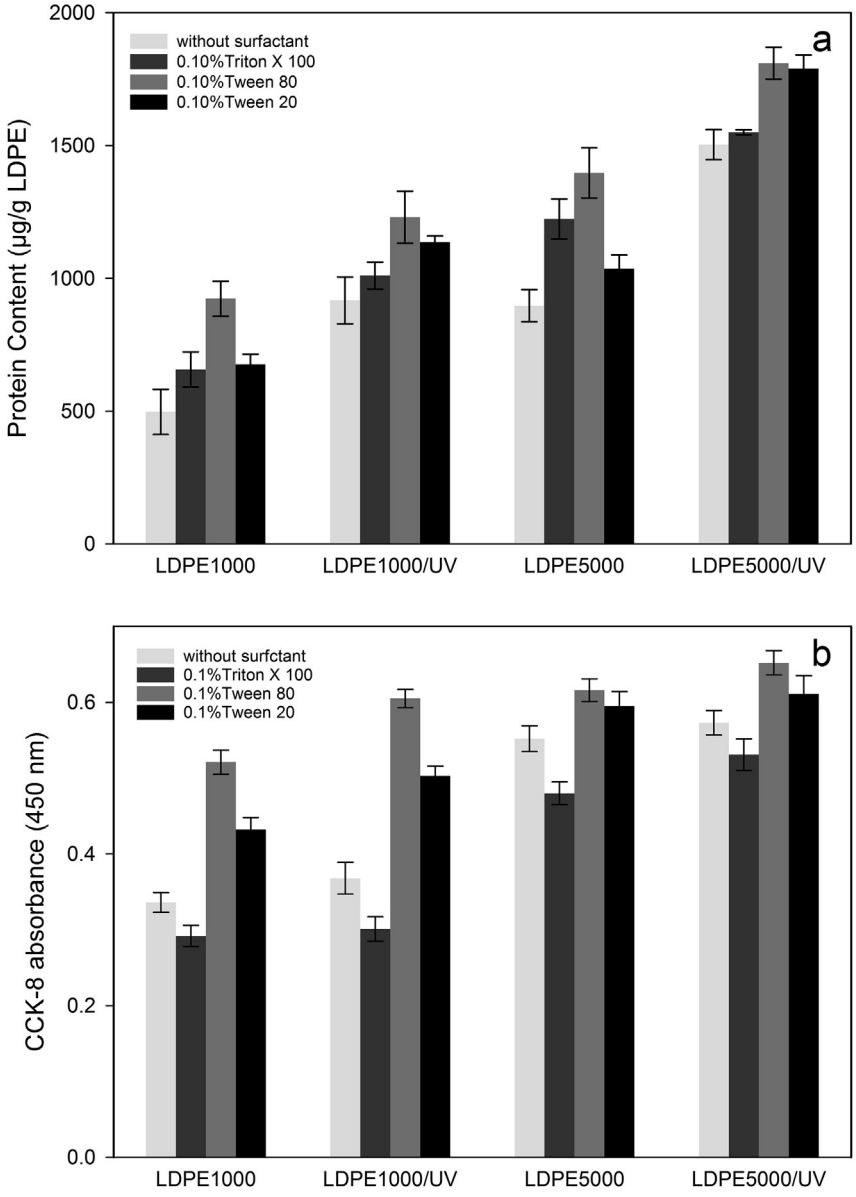

Fig. 3. The amount of protein (a) and viability (b) of the biofilm on LDPE MPs sampled from microcosm experiments with the pretreatment of UV-irradiation and the amendment of nonionic surfactant (Triton X100, Tween 80 or Tween 20 at a concentration of 0.10%).

diversity of the colonizing microbial taxa (Zettler et al., 2013; Debroas et al., 2017). Compared with untreated MPs, UV-pretreated MPs in the present study contained lower microbial taxa richness and had reduced within-sample diversity. For example, the ACE, Chao1, Shannon and Simpson indices of LDPE5000-associated biofilm were calculated to be 372 ± 48, 373 ± 49, 5.8 ± 0.3, 0.95 ± 0.01, which were decreased to 219 ± 11, 219 ± 20, 2.5 ± 0.2, 0.58 ± 0.03 in the LDPE5000/UV samples, respectively. β-diversity analysis using PCoA indicated that replicate samples generally clustered together and the UV-pretreated samples (LDPE5000/UV, LDPE1000/UV, LDPE5000/UV + Tw80 and LDPE1000/UV + Tw80) were separated from other samples by principal component 1 (Fig. 5). These results suggested that the change in surface roughness and hydrophobicity of microplastic that induced by UV-preirradiation was important factor shaping the LDPE MP-associated microbial community structure. To our knowledge very few studies have investigated the α- and β-diversity of bacterial communities colonizing MPs in term of surface properties, and the present study provided a first glimpse on this issue.

3.4. LDPE MPs as transport vector for pathogenic microorganisms from lake water

From the results of microbial community analysis, half of the top 20 most abundant genera were found to be the genera that contain pathogenic taxa (Fig. S6). They were *Comamonas*, *Agrobacterium*, *Brevundimonas*, *Acinetobacter*, *Sphingobacterium*, *Wautersiella*, *Chryseobacterium*, *Flavobacterium*, *Bacillus*, and *Clostridium* (Table S2).

Of them, the genus *Agrobacterium* contains important plant pathogens that can cause unorganized tissue growth or profuse abnormal root development (Otten et al., 2008). *Acinetobacter*, widespread in soil and water, is common infectious pathogen, and is emerging as problematic pathogens due to its capability to develop stable antibiotic resistance (Bhargava et al., 2010). *Acinetobacter baumannii*, the most important representative pathogen,

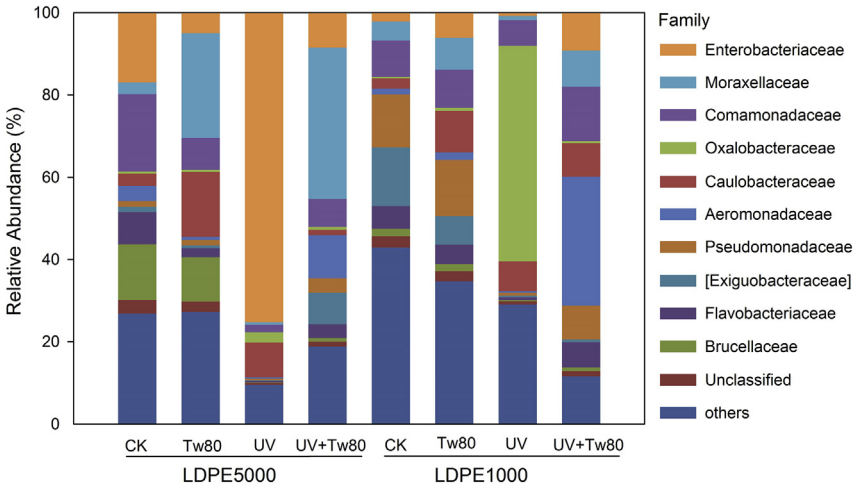

Fig. 4. Abundance profiles of the sequences affiliated with different bacterial families relative to the total number of sequences from the LDPE MPs. Sequences not classified to any known families are included as unclassified bacteria, and the bacterial families out of the top 10 are included as others (the ranking based on the average relative abundance).

Table 1
Indices of α-diversity in LDPE samples.

Treatment	Observed OTUs	ACE	Chao1	Shannon	Simpson
LDPE5000	371 ± 47	372 ± 48	373 ± 49	5.8 ± 0.3	0.95 ± 0.01
LDPE5000/UV	213 ± 15	219 ± 11	219 ± 20	2.5 ± 0.2	0.58 ± 0.03
LDPE5000 + Tw80	412 ± 30	417 ± 29	415 ± 32	5.6 ± 0.2	0.94 ± 0.01
LDPE5000/UV + Tw80	321 ± 24	326 ± 28	324 ± 26	5.0 ± 0.4	0.90 ± 0.03
LDPE1000	391 ± 7	396 ± 6	395 ± 8	5.1 ± 0.7	0.88 ± 0.07
LDPE1000/UV	295 ± 10	302 ± 13	296 ± 9	3.6 ± 0.2	0.72 ± 0.05
LDPE1000 + Tw80	466 ± 17	475 ± 15	469 ± 16	6.6 ± 0.04	0.98 ± 0.001
LDPE1000/UV + Tw80	330 ± 42	338 ± 46	336 ± 45	4.7 ± 0.6	0.88 ± 0.07

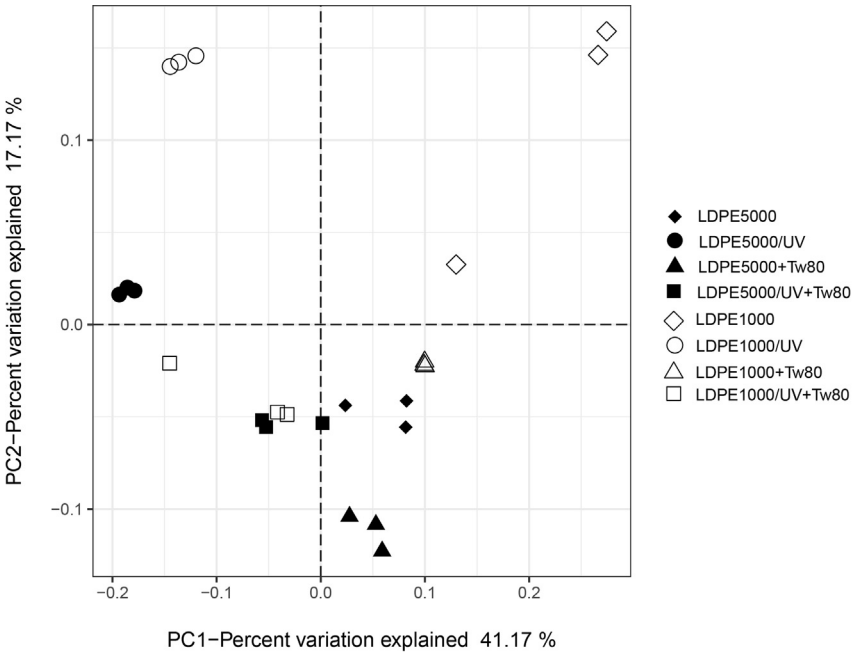

Fig. 5. Principal coordinates analysis (PCoA) of microbial communities from LDPE MPs based on the weighed unifrac distance.

can cause infections in the central nervous system, skin and soft tissue, and bone, but resistant to all known antibiotics have now been reported (Peleg et al., 2008). As nosocomial pathogens, the occurrence might be associated with the hospital near the lake where water used in the present study was collected. Belonging to the genus *Chryseobacterium*, *Chryseobacterium meningosepticum* and *Chryseobacterium indologenes* are found to cause incidence of healthcare-associated infections, and the infections involve

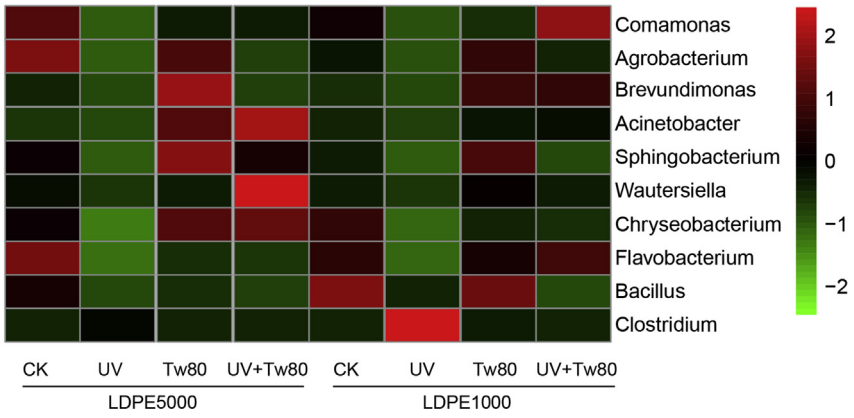

Fig. 6. A heatmap of the most abundant potential pathogens at the genus level recovered from LDPE MPs. Green denotes a low relative abundance across a taxon (row); red denotes a high relative abundance. The color key for the Z score indicates correspondence between the green-red coloring and standard deviations from the mean abundance of each taxon. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

bacteremia, pneumonia and meningitis, etc. (Bloch et al., 1997; Deng et al., 2015). Within the genus *Flavobacterium*, three *Flavobacterium* species (*Flavobacterium psychrophilum*, *Flavobacterium columnare*, *Flavobacterium branchiophilum*) are serious fish pathogens (Bernardet and Bowman, 2006). Especially, *Flavobacterium branchiophilum* is identified as the main causative agent of bacterial gill disease, which affects various cultured freshwater fish species worldwide (Wakabayashi et al., 1989). In lake water, such colonization of microplastic by fish pathogens is a great threat to fish safety because of the high ingestion of microplastic by fish. Without specific interpretation in the main text, the effects of other potential pathogens are described in detail in Table S2. To date, McCormick et al. (2014) provided the only in situ evidence for MP-colonizing pathogens in freshwater environments, and they identified the high abundance of family Campylobacteraceae and genera *Aeromonas*, *Arcobacter* and *Pseudomonas* that could contain pathogenic strains. The different predominance of potential pathogens on MPs in the present study might be related to the different source of freshwater, and those factors driving biofilm composition on MPs (Harrison et al., 2018) are needed to be investigated with reference to pathogens on MPs.

Fig. 6 is a heat-map that compared the abundance of potential pathogens that were found in the top 20 most abundant genera in all LDPE MPs samples. Results showed that LDPE1000/UV and LDPE5000/UV harbored less potential pathogens than other microplastic samples, while LDPE microplastics with surfactant presence were observed to be hotspots for potential pathogens. It has been suggested that organic aggregates may provide a favorable microhabitat for aquatic microbial pathogens (Lyons et al., 2010). The present study demonstrated that LDPE MPs could serve as microscopic island for harboring pathogenic microorganisms in lake water, this finding is important because this not only relates to the dispersal of pathogens but also involves pathogen accumulation through foodweb transfer in freshwater environments (Oberbeckmann et al., 2015a).

4. Conclusions

This study demonstrated that bacteria within environmental lake water can rapidly colonize LDPE MPs, and microbial colonization and biofilm formation on MPs surface was enhanced by the amendment of nonionic surfactants and the pretreatment of UV-irradiation. Proteobacteria, Firmicutes and Bacteroidetes were predominant phyla for all LDPE samples, but the phylogenetic composition and structure of microbial biofilms on LDPE MPs were affected by the surface chemistry and structure of LDPE MPs especially the surface roughness and hydrophobicity. The results also demonstrated that LDPE MPs harbored harmful microorganisms from lake water, and the surface chemistry and structure of MPs seemed to affect microbial pathogens colonization on MP surface. Although the present study was performed under controlled conditions, the information acquired is beneficial for assessing the natural diversity of microplastic-colonizing microbial communities, especially for subtropical region in developing countries.

Declarations of interest

None.

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Appendix. ASupplementary data

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