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Is there a significant difference in microbiota between water and microplastic surfaces in winter? The possibility of spreading offshore into the ocean

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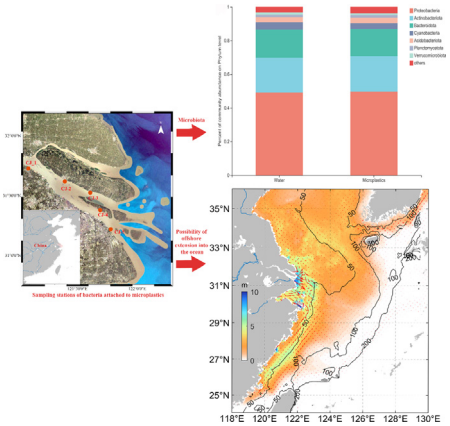
HIGHLIGHTS

- The bacteria in the surface MPs showed strong site-dependence in winter.
- No significantly different between MPs surface bacteria and planktonic bacteria
- The potential ecological risk of bacteria in MPs surface biofilm is low.
- Low possibility of bacteria attached to MPs entering the Pacific Ocean.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental problems caused by microplastics (MPs) are attracting global attention. The ecological risks of bacteria attached to MPs have not been studied in detail under low temperature conditions. Here, MPs in surface water were sampled in winter from the Changjiang (or Yangtze) River Estuary. The physical and chemical characteristics of the MPs were identified, and the diversity and species composition of bacteria on the surface water MPs were analyzed. Phenotypic prediction analysis was used to analyze the potential risk of bacteria in the biofilm on the surfaces of MPs. The main chemical composition in the MPs in the surface water were PP (polypropylene), PE (polyethylene), PS (polystyrene) and other light weight MPs. Sampling sites played a decisive role in the bacterial species composition. The potential plastic-degrading bacterium *Acinetobacter* and the potential pathogenic bacterium *Pseudomonas* showed significant differences across different sampling sites. Microbial communities on the surfaces of MPs in winter were not significantly different from planktonic bacteria in the water body. Phenotypic prediction results showed that bacteria on the surface of MPs had a marked capacity to form biofilms, but a low pathogenicity risk. Based on the results of biodiversity analysis and phenotypic prediction, the potential ecological risk of bacteria in biofilms on MP surfaces is lower at low temperatures. In addition, the numerical simulation results show that the possibility of bacteria attached to MPs from the Changjiang River entering the Pacific Ocean in winter is small. MPs attached bacteria in the Changjiang estuary have low ecological risk to the estuary and the Pacific Ocean in winter.

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1. Introduction

Global plastics production reached 368 million tons in 2019 (Plastics., 2020). Due to their lightness, ease of use, high mechanical strength, and resistance to oxidation and moisture, plastics are widely used in various industries. Improper management of damaged and discarded plastics causes them to enter the natural environment where they gradually decompose to MPs smaller than 5 mm. These MPs are called secondary MPs, while plastic particles produced smaller than 5 mm are called primary MPs (Ivleva et al., 2017). MPs are ubiquitous in the environment and they have been found in soils (Machado et al., 2018), highland areas (Zhang et al., 2021b), ocean surfaces (Li et al., 2021), air (Liu et al., 2020), and even in polar regions (Kanhai et al., 2018). The stable chemical properties of plastics mean they are difficult to degrade under natural conditions. Rivers are an important channel for MPs to enter the ocean. It is estimated that 1.15 to 2.41 million tons of MPs enter the ocean each year (Lebreton et al., 2017). MPs are similar in size to phytoplankton, making them vulnerable to ingestion by aquatic organisms such as fish and shellfish (Tanaka and Takada, 2016; Teng et al., 2019). High concentrations of MPs are harmful to the growth of juvenile oysters and the reproduction of adult oysters (Gardon et al., 2018; Thomas et al., 2020). There are many pollutants in the environment, such as heavy metals Pb (Abbas et al., 2022; Khalil et al., 2022; Rasool et al., 2022; Tauqeer et al., 2022). MPs may adsorb persistent organic pollutants and heavy metals (Feng et al., 2020). Besseling et al. (2017) showed that persistent pollutants adsorbed to MPs can accumulate in organisms after MPs are ingested. Ma and You (2021) researched the accumulation of MPs in the food web in the Baiyangdian Lake ecosystem and found that MPs can accumulate in the food chain, with cumulative effects as the trophic level increases; however, there is a time lag in the trophic level accumulation of MPs in the food web. MPs may eventually cause harm to humans (Rochman et al., 2015).

Microorganisms form extracellular polymers called biofilms by secreting extracellular proteins, polysaccharides, nucleic acids, and lipids. Bacterial biofilms serve to resist adverse conditions in natural environments (Flemming and Wingender, 2010). With the protection of biofilms, microorganisms can better survive than planktonic bacteria in adverse environmental conditions. Carpenter et al. (1972) was the first to discover that microbes attach to plastic surfaces. Bacteria, algae, and protozoa can attach to plastic surfaces (Zettler et al., 2013). Microbial attachment has been found on plastics, as well as on MPs (Harrison et al., 2014). Biofilms protect bacteria from ultraviolet radiation (Flemming and Wingender, 2010), which reduces the decomposition of MPs by sunlight. Plastic-degrading bacteria also exist on the surface of MPs (Harshvardhan and Jha, 2013; Urbanek et al., 2018). In addition, pathogenic microorganisms such as *Vibrio* and *Pseudomonas* also attach to the surface of MPs (Curren and Leong, 2019; Kesy et al., 2020). Species on the surface of plastics can also migrate long distances along with the plastics. The tsunami caused by the earthquake in Japan led to the appearance of 289 species along the coast of North America and Hawaii, which had originated in Japan; these species were mainly attached to the surface of plastic debris (Carlton et al., 2017). MPs are as difficult to degrade as plastic debris; the concentration of MPs in the environment is higher than that of plastic fragments, and they are widely distributed. The risk of species invasions caused by MPs is much higher than that of plastic fragments.

The Changjiang (Yangtze) River, the largest river in China, carries 9.24×10^{11} m^3 of fresh water into the Yellow Sea and the East China Sea each year. The Changjiang River is the largest source of fresh water to the western Pacific Ocean (Kang et al., 2013). MPs in the fresh water of the Changjiang River are discharged to the western Pacific Ocean along with the river water. There is still a lack of research on the ecological risk of microorganisms in the biofilm on the surface of MPs at low temperature. Here, five sampling stations were established to collect MPs in winter from the Changjiang River. The objectives were: 1. characterize MPs in the Changjiang River Estuary in winter; 2. identify regional differences in species composition in MP-attached bacteria; 3. identify the difference between microbiota in water and on MP surfaces in winter; 4. determine the potential ecosystem risks of MP-attached microorganisms at low temperatures, and 5. analysis of the likelihood of microorganisms attached to MPs surfaces reaching the ocean.

2. Sample collection

In January 2021, we sampled bacteria attached to MPs at five sampling sites along the Changjiang River Estuary (Fig. S1). The average annual winter temperature of the water body in the sampling area was 8 °C. The sampling longitude and latitude are shown in Supplementary Table 1. Samples were collected by the following methods at each sampling site.

2.1. MP sample collection

Individual MP particles are small and difficult to collect in situ. We improved the method of Jiang et al. (2018) by using an iron mesh with a diameter of 1 mm and placing it in a large aperture fishing net to retrieve MPs. MPs were collected from surface to depth of 30 cm water. Sampling is based on minimizing the influence of human factors and avoiding areas with high human activity. The length of MPs obtained were estimated by the number of holes in the iron screen occupied by MPs. The MPs were picked up with sterile tweezers. Then, each piece of MP was placed in a 15 mL sterile centrifuge tube. Fifteen MP samples were collected at each station. After labeling, the samples were stored in dry ice at −78 °C. When returned to the laboratory, they were stored in an −80 °C freezer until analysis.

2.2. Water sample collection

Water samples below 10 cm of the water surface were collected with a water sampler. Collected water was poured into a 1 L sterile bottle. Three samples were collected in parallel and placed in an ice box for temporary preservation. When returned to the laboratory, the water samples were filtered in a sterilization cabinet through a 0.22 μm filter membrane, then placed in a 50 mL sterile centrifuge tube for storage. Meanwhile, three samples of 1 L sterile water (ddH_2O) were filtered as control samples. These samples were stored in a freezer at −80 °C.

2.3. DNA extraction and PCR amplification

From the collected MP samples, three samples were selected for DNA extraction at each station. In addition, DNA was extracted from the water body of the filter membrane at each station. Microbial community genomic DNA was extracted from MPs and water samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to kit instructions. The extracted DNA was checked by 1 % agarose gel electrophoresis. The bacterial community was amplified by primers 515F (5′-GTGCCAGC MGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) for the highly variable region of bacterial 16S rRNA gene by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The amplification procedure was same as Dong et al. (2021). Each sample had three replicates.

2.4. Contamination prevention

The iron screen, net, tweezers, water sampler, and 0.22 μm filter membrane were sterilized in a high-pressure steam sterilizer (121 °C) before use. Three sterilized MP particles were soaked in water, and then put into a new centrifuge tube as MP control samples. DNA extraction and PCR amplification were carried out on the MP control samples. The results showed that no amplification band was found in MP control samples, which indicated that environmental water attached to MPs would not affect the results of bacteria attached to MP surfaces. In addition, DNA extraction and PCR amplification were also carried out on the water control sample (ddH_2O), and no amplification band was found. This showed that there was no contamination of samples during the experiment.

2.5. Illumina MiSeq sequencing

Using 2 % agarose gel extracted the sample PCR product. Amplicons were purified by AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified by Quantus™ Fluorometer (Promega, USA). Purified amplicons were sequences on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw 16S rRNA gene was quality control by fastp (https://github.com/OpenGene/fastp, version 0.20.0) (Chen et al., 2018) and spliced by FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.7) (Magoc and Salzberg, 2011) with the following principles: (1) Filter the bases with the quality value below 20 in the tail of reads, set a 50 bp window, if the average quality value in the window is lower than 20, cut off the back-end bases from the window, filter the reads with the quality control value below 50 bp, and remove the reads containing N bases; (2) On the basis of the overlap relationship between PE reads, paired reads are spliced into a sequence with a minimum overlap length of 10 bp; (3) The maximum mismatch ratio allowed in the overlap region of the spliced sequence is 0.2; (4) Distinguish samples according to barcode and primers. The allowable mismatch number of barcode is 0, and the maximum primer mismatch number is 2. On the basis of using UPARSE software (http://drive5.com/uparse/, version 7.1), according to 97 % OTU on sequence of similarity clustering and eliminate chimeras (Edgar, 2013; Goebel, 1994). Each sequence was annotated for species classification by RDP classifier (Wang et al., 2007) (http://rdp.cme.msu.edu/, Version 2.2), and compared with Silva 16S rRNA database (Silva v138), setting the comparison threshold to 70 %.

2.6. Physical parameters and composition of MPs

The MPs after DNA extraction were photographed using a stereoscopic microscope (Leica M165FC, Germany). The length and perimeter of the MPs were measured using Image J software. A Micro-Fourier Transform Infrared Spectrometer (μ-FT-IR) (Thermo Nicolet iN10 MX, USA) was used to determine the chemical composition of the MPs. The degree of matching between the MP component samples and the plastic component in the database was set to be >70 % by comparing with the Omnic 9 software and Omnic spectral library.

2.7. Data processing

The map of the sampling station was drawn with Matlab 2019. ANOVA (Analysis of Variance) was tested with SPSS One-way ANOVA. A p-value < 0.05 was set as the significance level for all analyses, and α-diversity analysis was carried out using mothur (version v. 1.30.2) index analysis. The OTU similarity level was used for index assessment at a threshold of 97 %. COG (Clusters of Orthologous Groups) functional classification statistics was used to standardize the OTU abundance table through PICRUSt (PICRUSt stored COG information corresponding to Greengene ID). The influence of the copy number of the 16S marker gene on a species genome was removed. Through the Greengene ID corresponding to each OTU, the COG family information corresponding to OTU was obtained and the abundance of each COG was calculated. According to the information in the COG database, the description information of each COG and its functional information can be parsed from EggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups http://eggnog.embl.de/) database to obtain the functional abundance spectrum. LEfSe (Linear Discriminant Analysis Effect Size) multilevel discriminant all-against-all (stricter) analysis was used with LEfSe species differences (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload). Bugbase phenotypic prediction was conducted according to Ward et al. (2017). Other data processing and mapping used the R language (version 3.3.1). The ecological risk of MPs to the ocean was assessed by analyzing the extension of the Changjiang River freshwater. MPs enter the ocean with river freshwater. The distribution of MPs in the ocean can be estimated by calculating the total volume of river freshwater. The fresh water thickness distribution of the Changjiang River freshwater was based on the model of The Regional Ocean Modeling System (ROMS) by Wu et al. (2021). Zhang et al. (2012) method was adopted to calculate river freshwater. Freshwater thickness of Changjiang River was calculated as

$$h = \int_{-H}^{\eta} dyedz, \tag{1}$$

where dye is the river water concentration, η is sea surface high and H is the total water depth. Where pure seawater dye content is 0, while pure freshwater dye is 1. The physical explanation for the freshwater thickness is that if we "decompose" the water column so that all freshwater is above the pure seawater, the thickness of the freshwater layer is the freshwater thickness. For the dye thickness, h is the total freshwater of Changjiang River at each grid in meter. And h times the area is the total volume of freshwater in this grid point.

$$\vec{Q} = \int_{-H}^{\eta} \vec{v}\, dyedz, \tag{2}$$

where $\vec{Q}$ is the volume transport of freshwater, and $\vec{v}$ is the vector velocity. In this study of the Changjiang River, we calculated the monthly mean freshwater thickness and vertically integrated transport. The environmental parameters in the model are described in the Supplementary materials.

3. Results

3.1. Chemical composition and physical characteristics of MPs

The chemical composition and physical parameters of the MPs after DNA was extracted and sequenced are shown in Supplementary Table 2. There were 8 (53.3 %) polyethylene (PE) MPs, 5 (33.3 %) polypropylene (PP) MPs, 1 (6.7 %) polystyrene (PS) and 1 (6.7 %) polyethylene- polypropylene (PE-PP) MP. The length distribution of MPs ranged from 1.8 mm to 4.8 mm. The main types of plastics were fragmental MPs (Fig. 1); only one piece of linear MP was recorded (Fig. 1H). Obvious breakage and fading were observed on the surface of the MPs (Fig. 1E, G).

3.2. Analysis of microbial diversity in MP biofilms

Among the five sampling sites in the Changjiang River, the dilution curves of the 16S rRNA gene high-throughput sequencing showed a stable state in water planktonic bacteria and in bacteria in biofilm on the surfaces of MPs, thus, indicating good accuracy and depth of sequencing (Fig. S2). CJ-1, CJ-2, CJ-3, CJ-4, and CJ-5 obtained 1199, 1723, 1819, 1745, and 1175 OTUs bacteria attached to MP surfaces, respectively, which showed an increasing, then decreasing trend from estuary to ocean. Non-metric multidimensional scaling (NMDS) was used to analyze MP-attached bacteria and planktonic bacteria in water at different sampling sites (Fig. 2A). The bacteria in the surface water of CJ-1-W, CJ-2-W, CJ-3-W, CJ-4-W, and CJ-5-W showed good aggregation based on sampling stations, and the bacteria in the surface biofilms of CJ-1-P, CJ-2-P, CJ-3-P, CJ-4-P, and CJ-5-P MPs in the five sampling sites showed equally strong site-dependence. Based on the analysis of bacteria in biofilms on the surfaces of MPs and planktonic bacteria in water, 2968 OTUs of bacteria in water and 2944 OTUs on the surfaces of MPs were found in the Changjiang River Estuary. The OTUs shared by MP surface biofilms and water accounted for 86.7 % of the OTUs on the surfaces of MPs (Fig. S3). The α-diversity of planktonic bacteria and bacteria on the surfaces of MPs was analyzed by bacterial community richness (Sobs, Ace, Chao), bacterial community evenness (Shannon and Simpson), and bacterial community coverage (Coverage). The bacterial diversity index was tested by ANOVA. Although bacteria in the biofilms were different from the water body, the richness, evenness, and coverage of the microbial communities on the surfaces of MPs in winter were not significantly different from planktonic bacteria in the water body ($p > 0.05$ %) (Fig. S4). Furthermore, partial least

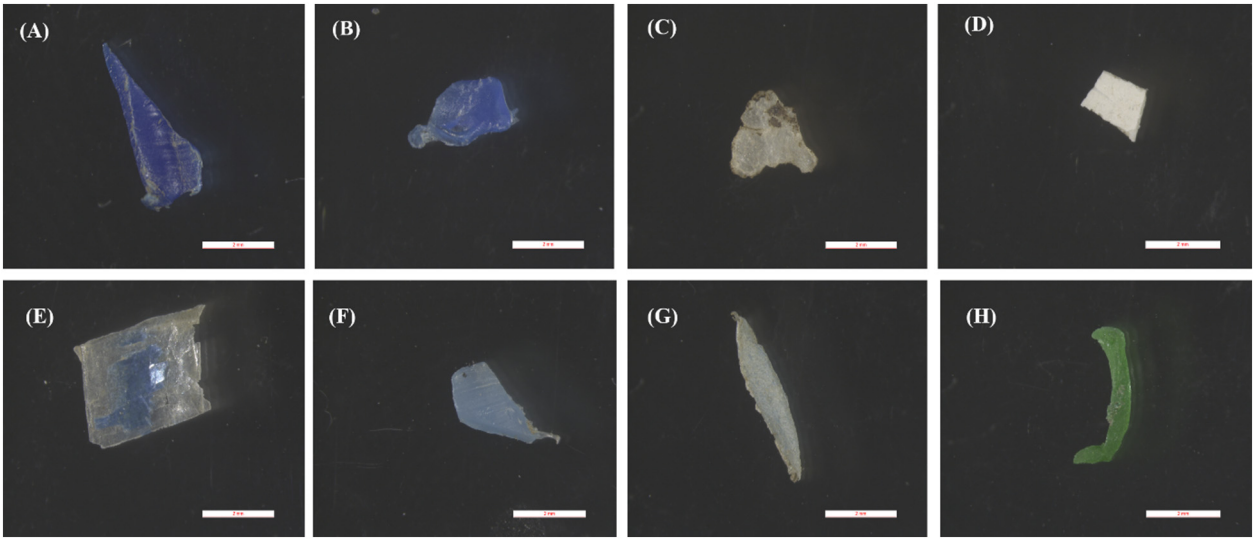

Fig. 1. Images of typical MPs in Changjiang River (size unit: 2 mm).

squares discriminant analysis (PLS-DA) was used to analyze the bacteria attached to the surfaces of MPs and plankton in the water. Bacteria on the surface of MPs and planktonic bacteria in water both showed good aggregation. It was confirmed that there were differences between bacteria on the MPs and planktonic bacteria in water (Fig. 2B).

3.3. Structure and taxonomic composition of MP biofilm communities

The characteristics of microbial communities on the surfaces of MPs were analyzed at phylum and family levels in the Changjiang River Estuary sample stations. Proteobacteria (43.6 %–56.5 %), Actinobacteriota (18 %–22.8 %), and Bacteroidota (14.5 %–20.7 %) were the main phyla on the surface of MPs at the phylum level. From CJ-1 to CJ-5, Proteobacteria on the surfaces of MPs increased gradually from fresh water to seawater, while Cyanobacteria decreased gradually (Fig. 3A). Comamonadaceae (16.5 %–33 %), Sporichthyaceae (9.6 %–15.9 %), Iliumatobacteraceae (5.2 %–8.9 %), and Flavobacteriaceae (4.1 %–7.4 %) were the main families in MP surface-attached bacteria. In contrast to the number of OTUs on the MP surfaces, the dominant species Comamonadaceae showed a trend of first decreasing and then increasing from the river to offshore. Pseudomonadaceae were detected on MPs (Fig. 3B). Based on the analysis of bacteria attached to MP surfaces and plankton in water, we found that the plankton bacteria were highly similar to those on MP surfaces at phylum and family level (Fig. 4).

4. Discussion

4.1. MPs and surface-attached bacteria

PE was the main chemical composition of MPs in the Changjiang River Estuary, which is consistent with previous studies on the composition of MPs in the surface water of Changjiang (Yangtze) River Estuary (Zhao et al., 2019). Influenced by weathering and other factors in water, MPs fade into white or transparent plastics (Fig. 1E, G), resulting in most MPs in the ocean being transparent or white (Li et al., 2021; Wang et al., 2020a, 2020b, 2020c). The number of bacterial OTUs in water bodies and MP surfaces in the Changjiang River Estuary in winter was lower than that in water bodies and MP surfaces in the Pearl River Estuary (Yang et al., 2021). It may be that the low temperature in winter in the Changjiang River Estuary is not conducive to microbial growth. Bacteria in the biofilm on the surfaces of MPs showed obvious regional specificities (Fig. 2A). Feng et al. researched biofilms on MP surfaces in cultured coral

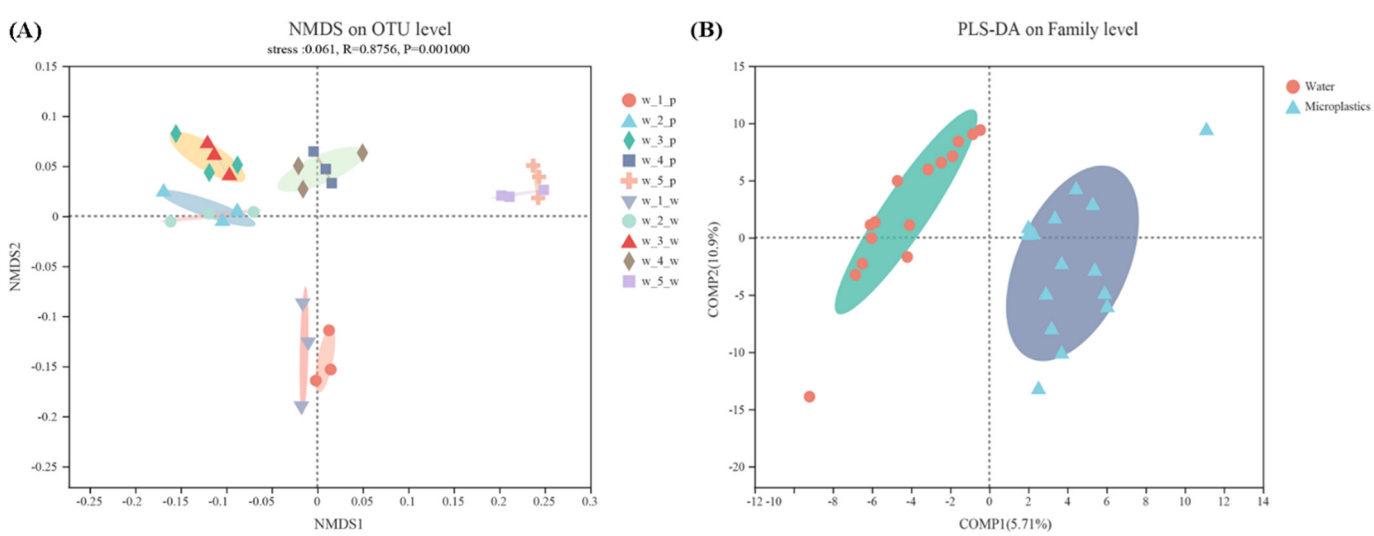

Fig. 2. Analysis of bacteria in biofilm on the surface of MPs and planktonic bacteria in water (A: NMDS analysis of water planktonic bacteria and bacteria attached to MP surfaces at different sampling stations, B: PLS-DA analysis of bacteria on the surface of MPs and water planktonic bacteria).

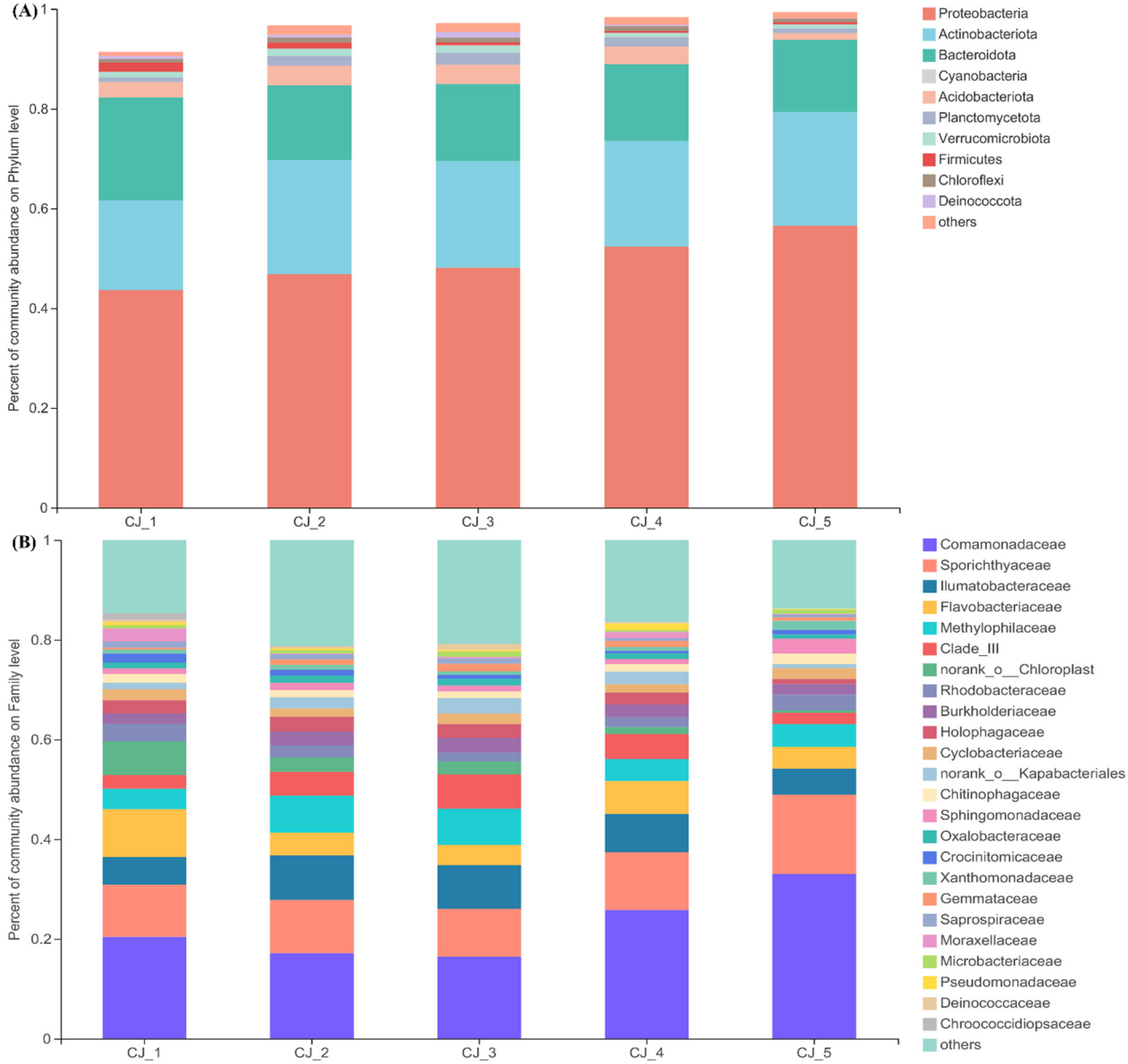

Fig. 3. Distribution of phylum and family level bacteria attached to MPs at different sampling stations in the Changjiang River Estuary in winter (A: Bacteria attached to MPs in the Changjiang River Estuary distributed at the phylum level, B: Bacteria attached to MPs in the Changjiang River Estuary distributed at the family level).

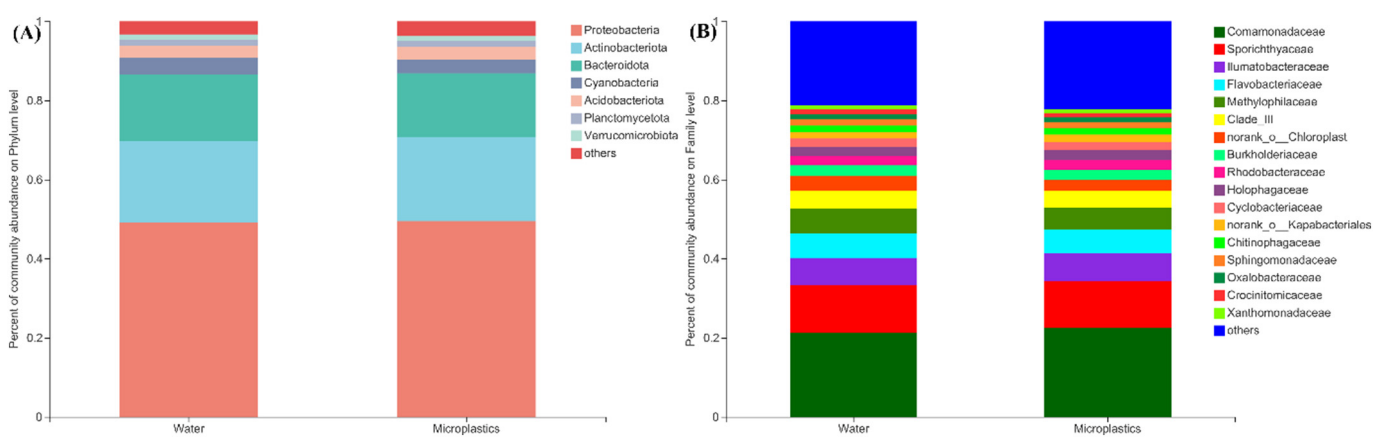

Fig. 4. Phylum and family species analysis of planktonic bacteria and bacteria attached to MP surfaces in water (A: Phylum species analysis of planktonic bacteria and bacteria attached to MP surfaces in water, B: Family species analysis of planktonic bacteria and bacteria attached to MP surfaces in water).

areas. It was found that microbial species in the biofilms on MP surfaces were affected by the surrounding water environment (Feng et al., 2020). The research of Li et al. on the formation of biofilm on the surface of MPs in Zhoushan Islands also showed that the microbes in the biofilm on the surface of MPs were mainly from seawater and the chemical composition of MPs had no significant effect on the species in the biofilm (Li et al., 2020). We analyzed bacteria on the surface of MPs with different chemical components from different sampling sites. It was found that the chemical composition of MPs did not influence the species on the MPs surface biofilm (data not shown). However, Frere et al. found significant differences in surface MP-attached bacteria between different compositions of MPs in Brest Bay, France (Frere et al., 2018). Therefore, more research is needed to determine whether the chemical composition of MPs affects the species of microorganisms on the surface.

In this study, the α diversity of bacteria attached to MP surfaces at different sampling points did not show significant differences with planktonic bacteria. Species analysis further proved that the bacterial species attached to the surfaces of MPs in winter were highly similar to water bodies at the phylum and family levels in the Changjiang River Estuary. In previous studies, the bacteria in the biofilm on the surface of MPs were obviously different from the planktonic bacteria in water (Li et al., 2020; Wang et al., 2020b). Research has shown that the ability to form bacterial biofilms is weak at low temperatures (Han et al., 2016). Their research showed that bacteria in biofilm on the surfaces of MPs proliferated slowly and could

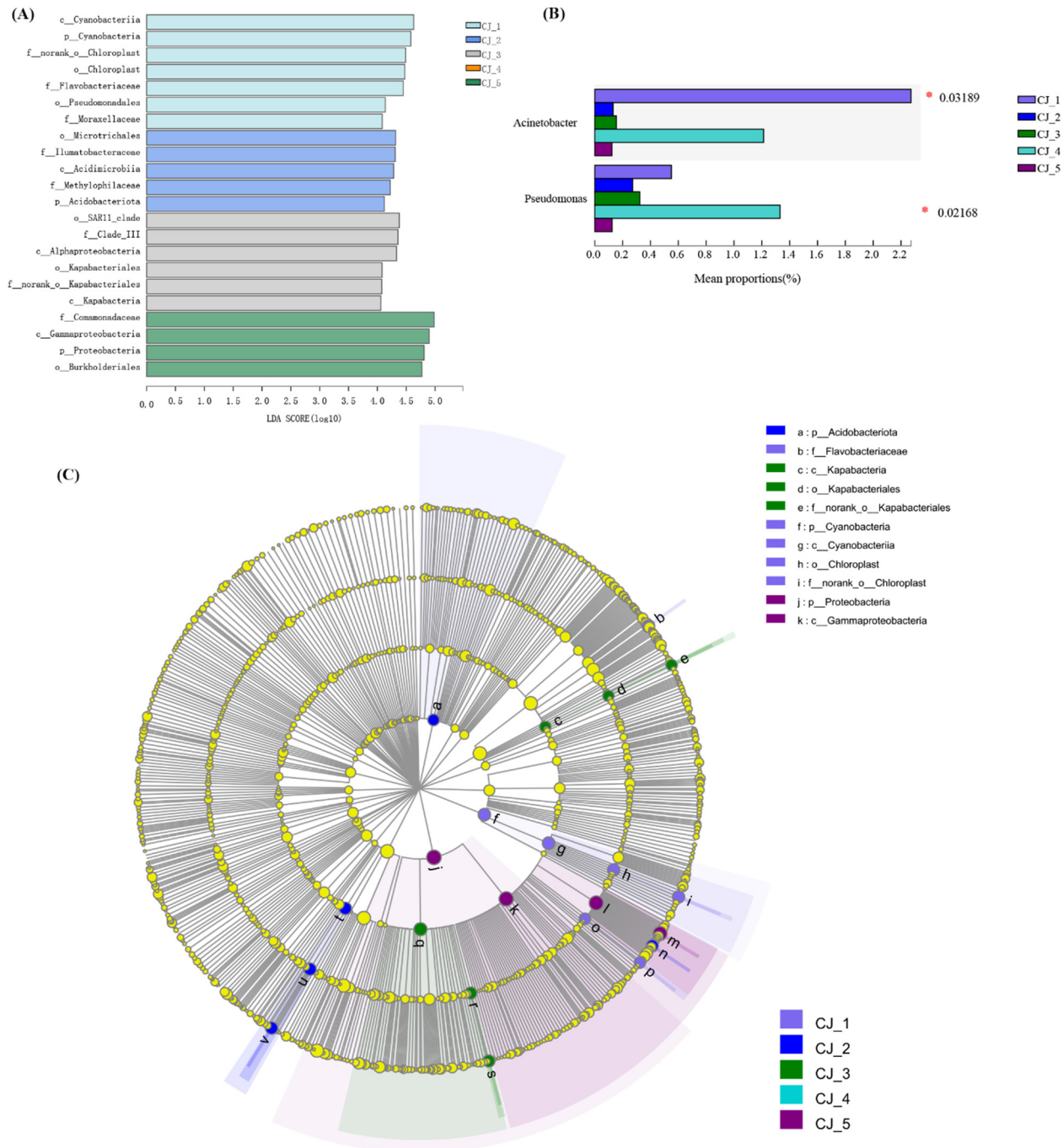

Fig. 5. Analysis of species on the surface of MPs at different sampling sites (A: LDA value was set to 4 for the analysis of different bacterial species on MP surfaces at different sampling sites, B: Analysis of potential plastic degradation bacteria *Acinetobacter* and potential pathogenic bacteria *Pseudomonas* at different sampling sites on MP surfaces, C: LEfSe multi-level species hierarchy tree diagram at different sampling sites on MP surfaces).

not form a dense biofilm at low temperature, which may explain our findings that the bacterial species compositions in biofilm on the surfaces of MPs was no different from the planktonic bacteria.

Proteobacteria and Bacteroidetes were shown to be the main phyla of bacteria attached to plastic surfaces in northern Europe and tropical coastal regions (Oberbeckmann et al., 2014; Curren and Leong, 2019). Proteobacteria and Bacteroidetes may play an important role in the early colonization of MPs (Jones et al., 2007). Proteobacteria, Actinobacteriota, and Bacteroidota on the surface of MPs were dominant at the phylum level in this study. The same results were found in the surface biofilm of MPs in coral areas and sediments (Feng et al., 2020; Zhang et al., 2021a). We found a high proportion of Actinobacteriota in the biofilm of MPs in Changjiang River Estuary, which is consistent with previous research on MPs in Chongming Island (an island in Changjiang River Estuary) (Jiang et al., 2018). The retention of nutrients in the water caused by the tides in the Changjiang River Estuary may be related to the high proportion of Actinobacteriota.

4.2. Effects of sampling site on bacteria at the surface of MPs

The LDA (Linear Discriminant Analysis) value was set to 4 to analyze the species with significant differences on the surface of MPs at different sampling sites. Compared with other sampling sites, there were four species with significant differences in CJ-1 (Fig. 5A). No species were found to be significantly different from those at other sampling locations at CJ-4. From the LEfSe multilevel species tree diagram (Fig. 5C) of different sampling sites, there were differences at phylum level at CJ-1, CJ-2, and CJ-5. CJ-1, CJ-2, CJ-3. CJ-5 showed differences in class, order, and family. Cyanobacteria showed obvious differences in CJ-1 compared with other sampling sites. Cyanobacteria are primary producers in water (Borgendahl and Westman, 2007). The bacteria attached to the surfaces of MPs at CJ-1 showed strong primary productivity. Potentially plastic-degrading Moraxellaceae showed significant differences at the CJ-1 station (Fig. 5A). The Kruskal-Wallis H test was used to analyze *Acinetobacter*, a potential plastic-degrading bacterium in the Moraxellaceae. Significant differences were found in *Acinetobacter* on MP surfaces at different sampling stations ($p = 0.0319$) (Fig. 5B). *Acinetobacter* shows great potential in plastic degradation. *Acinetobacter* could degrade PS (Wang et al., 2020a, 2020b, 2020c). *Acinetobacter* sp. and *Bacillus* sp. have the ability to efficiently degrade PE under co-culture conditions, with *Acinetobacter* sp. being the dominant bacteria in the co-culture (Yin et al., 2020). Di-2-ethylhexyl phthalate (DEHP) could be degraded by *Acinetobacter* sp. SN13 (Xu et al., 2017). *Acinetobacter gerneri* P7 shows good biodegradation of polyurethane (PU) (Howard et al., 2012). *Acinetobacter pitti* was able to degrade low density polyethylene (LDPE) films by 26.8 % in 4 weeks (Montazer et al., 2018). Potential plastic-degrading *Acinetobacter* bacteria were present at five stations in the present study. Research on *Acinetobacter* is potentially useful to solve pollution caused by MPs. *Pseudomonas* is widely distributed in the environment, and some *Pseudomonas*, such as *Pseudomonas aeruginosa*, can cause disease in humans or animals (Garrity et al., 2005). *Pseudomonas* were widely distributed on the surface of MPs at all sampling sites in this research, which is consistent with previous research

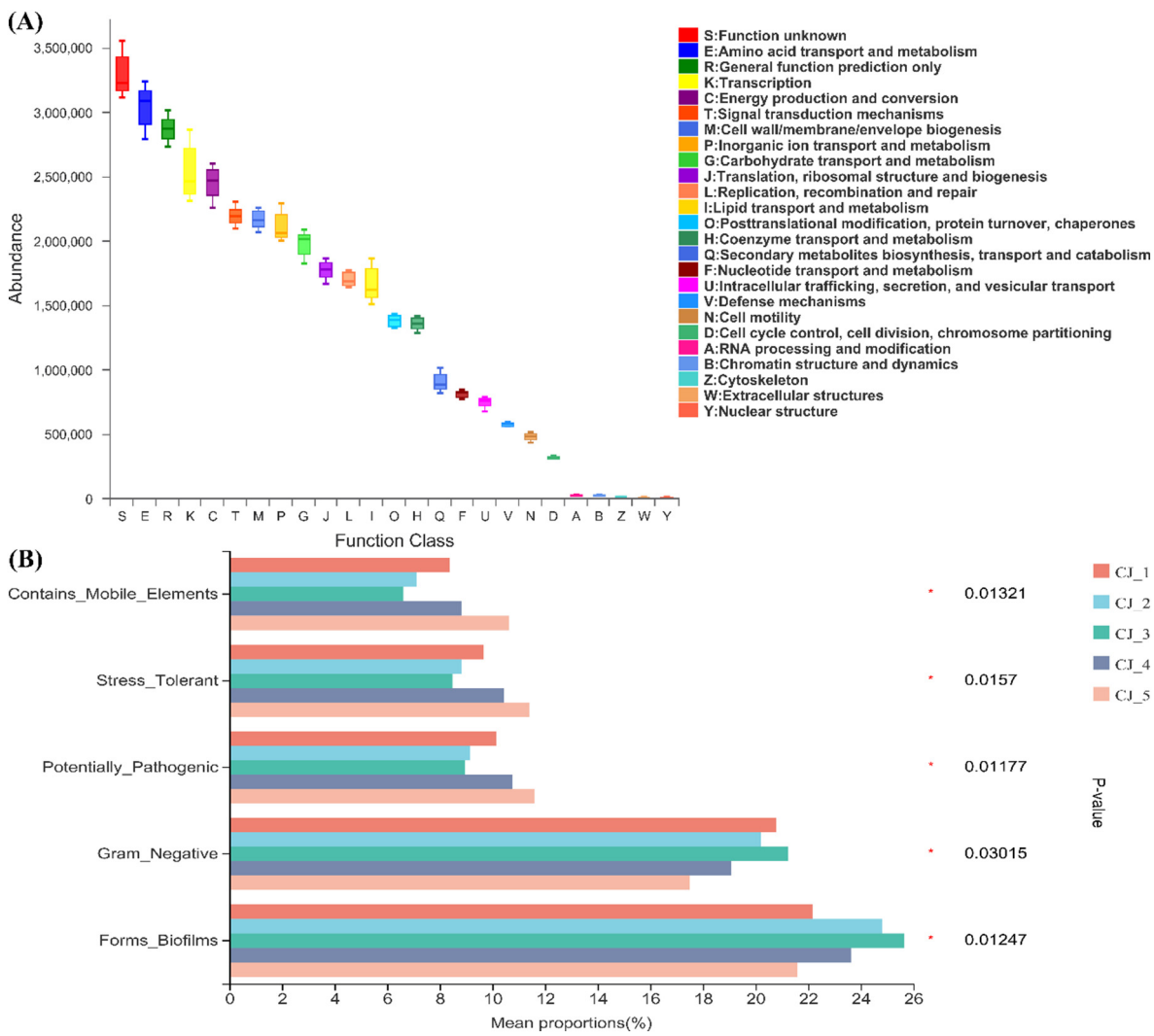

Fig. 6. Metabolic and BugBase phenotypes predictive in MP surface biofilm bacteria (A: COG metabolic analysis, B: Phenotypic predictive analysis).

(Hu et al., 2021; Shi et al., 2020; Wu et al., 2020). *Pseudomonas* on MP surfaces at different sampling sites showed significant differences (p = 0.0217) (Fig. 5B). Curren et al. showed that *Pseudomonas* is a potential pathogenic bacterium on the surface of MPs (Curren and Leong, 2019). Compared with natural materials, MP surfaces are capable of enriching pathogenic *Pseudomonas* (Wu et al., 2019). As pathogenic *Pseudomonas* is widely distributed in the surface biofilm of MPs in the environment, and MPs could enrich pathogenic *Pseudomonas*, more research should be conducted on the ecological risks caused by the enrichment of pathogenic bacteria on MP surfaces.

4.3. Metabolic and potential risk of biofilm attached to MP surfaces

The metabolic functions of bacteria attached on the surfaces of MPs were analyzed through COG (Fig. 6A). Amino acid transport and metabolism, transcription, energy production and conversion, signal transduction mechanisms, cell wall/cell membrane/envelope biogenesis, inorganic ion transport and metabolism, and carbohydrate transport and metabolism were the main metabolic processes on the surface of MPs. The main metabolic pathways of bacteria on the surface of MPs were related to the standard metabolism of bacteria in winter at the Changjiang River Estuary. The main reason for this phenomenon may be the low temperature in winter, which restricts the proliferation of bacteria, making bacteria retain a basic metabolism. Bacteria attached to the surfaces of MPs have a strong ability to form biofilm (>20 %) (Fig. 6B). The biofilm formation ability of bacteria on surface MPs in different sampling stations increased and then decreased and showed significant differences. Cotains_mobile_elements, Stress_tolerant and Potentially_Pathogenic showed opposite trends in biofilm formation, and there were significant differences among different sampling stations. The results showed that stress tolerant and potentially pathogenic might be weakened when bacteria form biofilms. By analyzing the α diversity and the differences between the bacteria in the biofilm on the surface of MPs and those in water, we demonstrated that the bacteria in the biofilm on the surface of MPs are less harmful in the natural environment at low temperature.

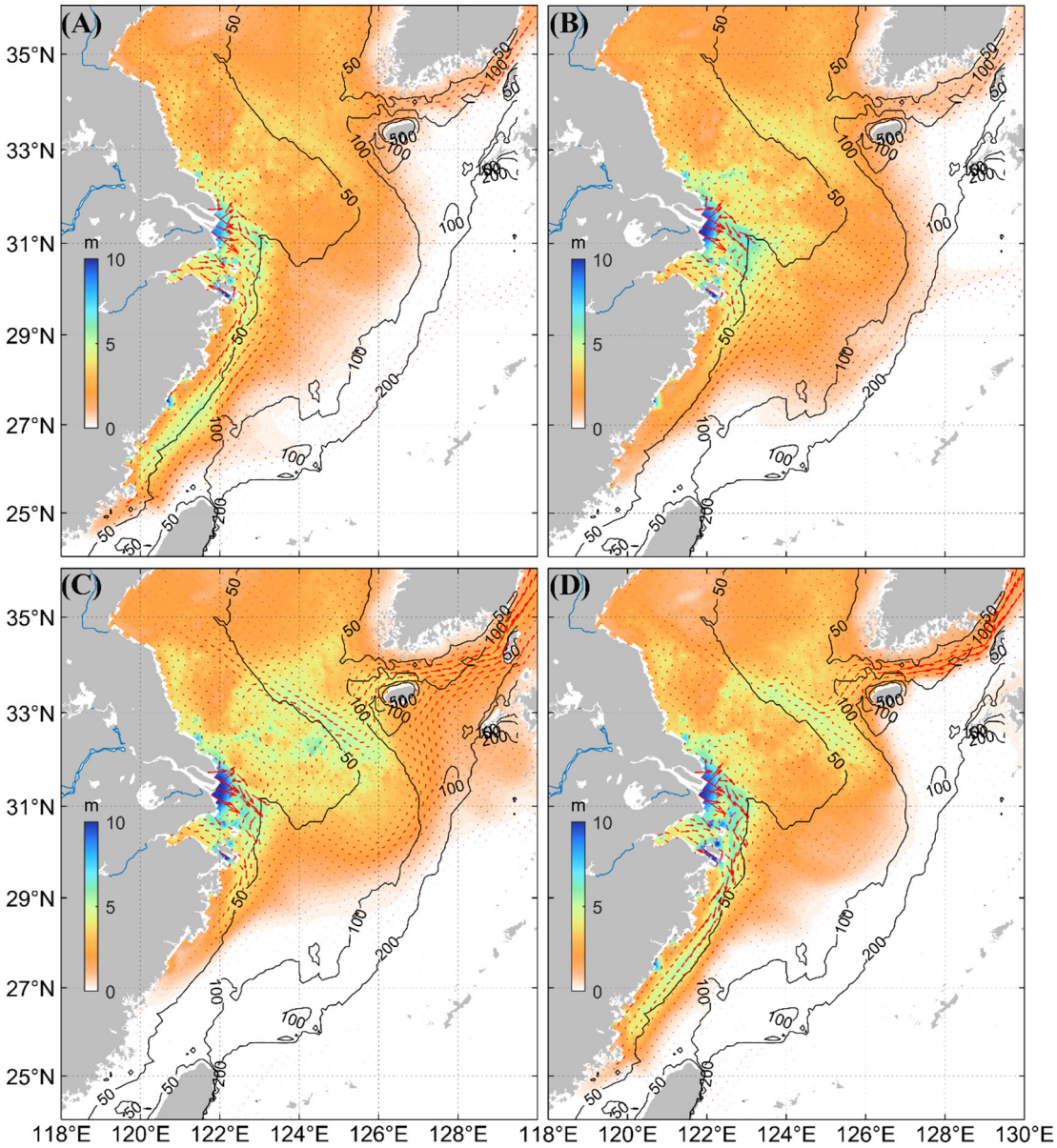

Fig. 7. Calculation of Changjiang River freshwater thickness in winter (A), spring (B), summer (C), and autumn (D). This reflects the likelihood of bacteria attached to MPs entering the ocean (Black lines are isobath lines and arrow size represents flow rate and concentration).

4.4. The possibility of offshore extension into the ocean

The transport of bacteria attached to the surface of MPs to the Pacific Ocean was evaluated by analyzing the distribution of freshwater in the Changjiang River. In order to analyze the possibility of dispersal from the coast to the ocean, a numerical model was used to simulate the Changjiang River freshwater. Also, the distribution of freshwater in the Changjiang River was tracked by adding passive tracers (dyes) to the river. Using a vertical integral passive tracer, the thickness of the freshwater layer in the Changjiang River was calculated, which represents the total amount of freshwater in the region from surface to bottom. The thickness of the Changjiang River freshwater in different seasons is shown in Fig. 7. The Changjiang River freshwater mainly enters the East China Sea and the Yellow Sea and expands in different directions due to the influence of wind and circulation. In summer, the south wind prevails, and the freshwater expands to the northeast. At this time, a large volume of freshwater in the Changjiang River enters Tsushima Strait, and part of it enters the western Pacific Ocean with Kuroshio. In winter, the north wind prevails, and the freshwater of Changjiang River expands southward, but mainly concentrates within the 50 m isobath, which can reach the Taiwan Strait. In spring and autumn, a lower volume of water enters the Pacific Ocean, mainly into the Sea of Japan. From the analysis of the distribution of the Changjiang River freshwater, it can be found that, in summer, the bacteria attached to MPs in the freshwater are highly likely to enter the Pacific Ocean. During winter, spring, and autumn, less Changjiang River freshwater enters the Pacific Ocean, and there is a lower risk that the bacteria attached to MPs will enter the Pacific Ocean.

5. Conclusion

The main MPs found in the surface water of the Changjiang River Estuary in winter were light weight MPs. The species composition of bacteria attached to MP surfaces was closely related to the planktonic bacteria in the surrounding water environment. The bacteria in the biofilm on the surface of MPs do not show significant differences with those in water under low temperature. The metabolism of bacteria maintained normal life activities due to the low temperatures in this season. There were potential plastic-decomposing bacteria on the surface of MPs in winter in the Changjiang River Estuary. Further research on plastic-decomposing bacteria could contribute to the resolution of the problem of MP pollution. In addition, although a small number of potential pathogenic bacteria were found on the surface of MPs at different sampling stations, because the bacteria in the biofilm on the surfaces of MPs are highly similar to those in water bodies, the attachment of biofilm on the surface of MPs at low temperature does not cause a high ecological risk. The numerical simulation results show that the possibility of MP-attached-bacteria from the Changjiang River entering the Pacific Ocean in winter is small. This study showed that MPs attached bacteria in the Changjiang estuary have low ecological risk to the estuary and the Pacific Ocean in winter.

CRediT authorship contribution statement

Xuri Dong: methodology, data analysis, preparation of the original draft. **Lixin Zhu:** research concept, editing the manuscript. **Ruiming Wu:** data analysis, ROMs model. **Changjun Li:** data curation. **Daoji Li:** supervision, resources, methodology, funding.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159769.

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