

Early stage of biofilm assembly on microplastics is structured by substrate size and bacterial motility

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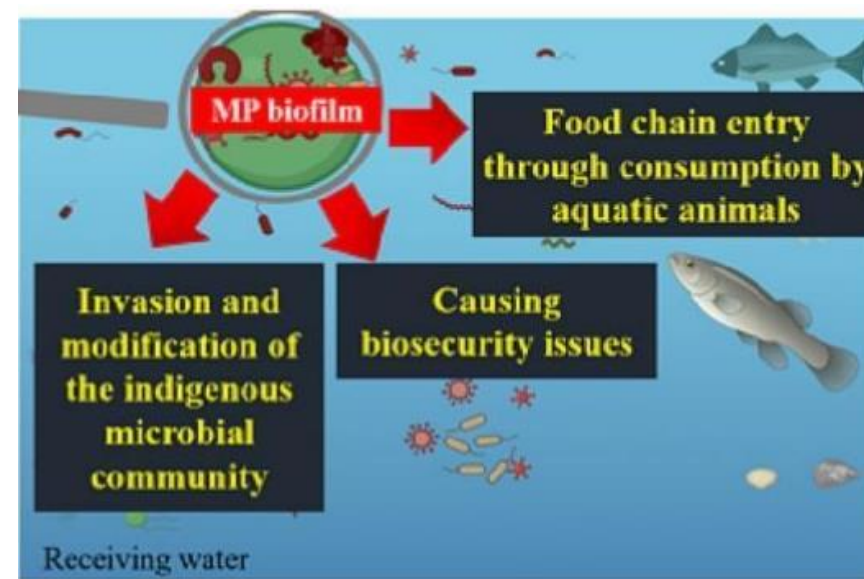


Peng Qin, Han Cui, Panxin Li, Shuaitao Wang, Shen Fan, Jie Lu, Meng Sun, et al. 2023. Early stage of biofilm assembly on microplastics is structured by substrate size and bacterial motility. *iMeta* e121.

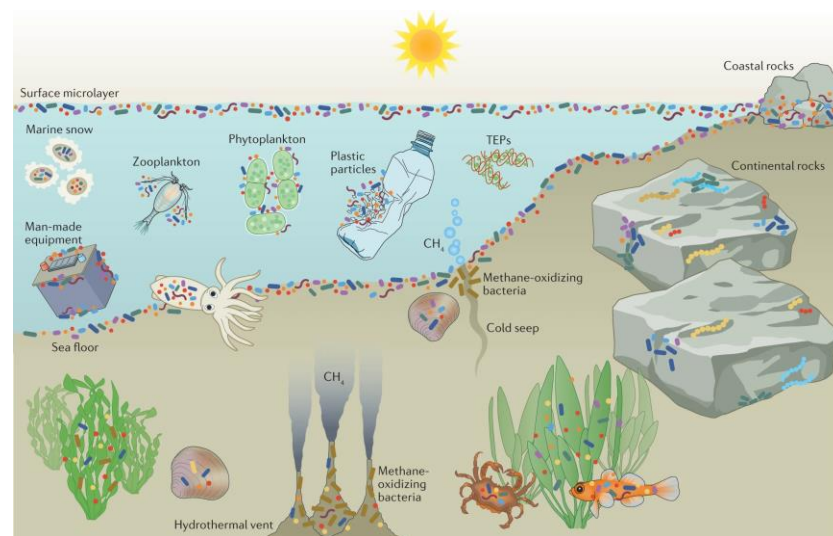
<http://doi.org/10.1002/imt2.121>

The biofilm communities on the surface of marine microplastics have important environmental impacts

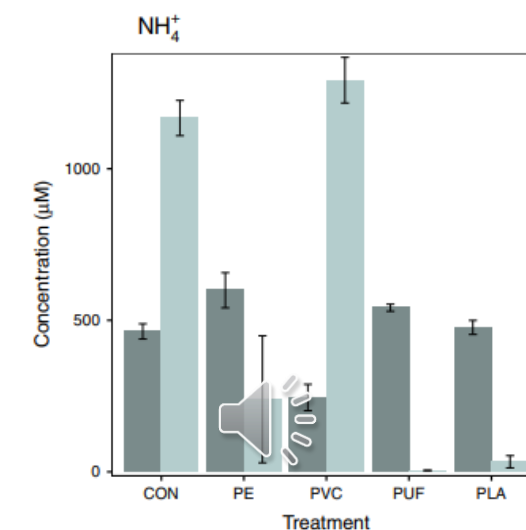
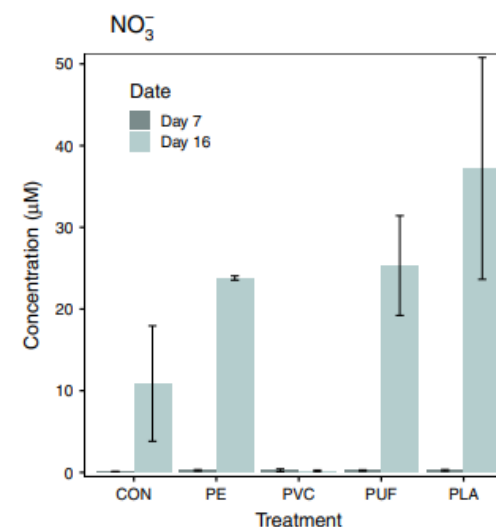
- Microplastic particles (MPs) are defined as plastic fragments with a diameter <5 mm and have become one of the most serious threats to marine ecosystems;
- The hydrophobicity on the surface of MPs promotes the settlement of bacteria and the formation of biofilms. The biofilm on the surface of MPs is defined as "plastisphere", representing a special type of biofilm;
- The biofilm on MPs has environmental impacts such as promoting the diffusion of MPs, influencing element cycling in marine ecosystems, and causing species invasion.



Rummel et al. 2017. Environ. Sci. Technol. Lett..



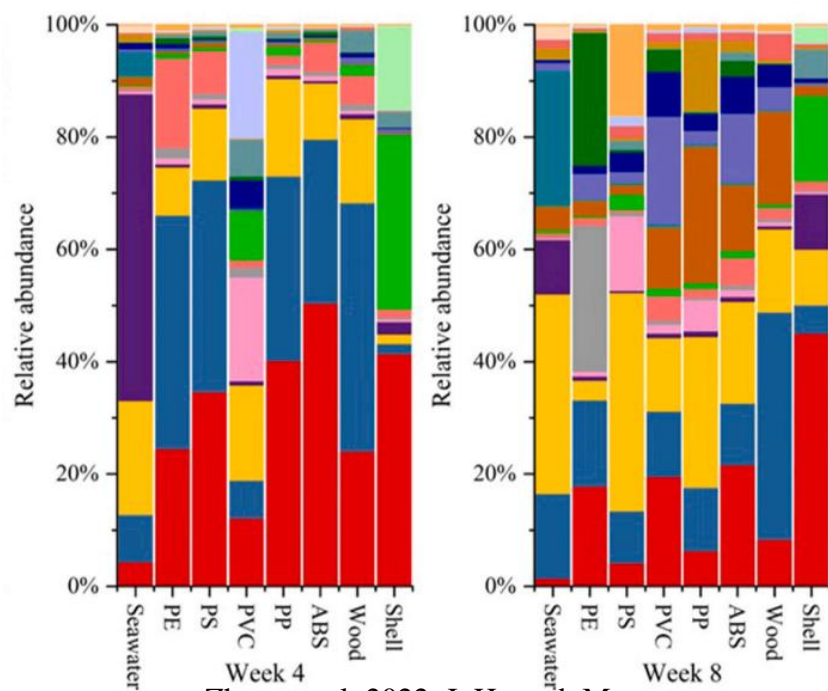
Qian et al. 2022. Nat. Rev. Microbial.



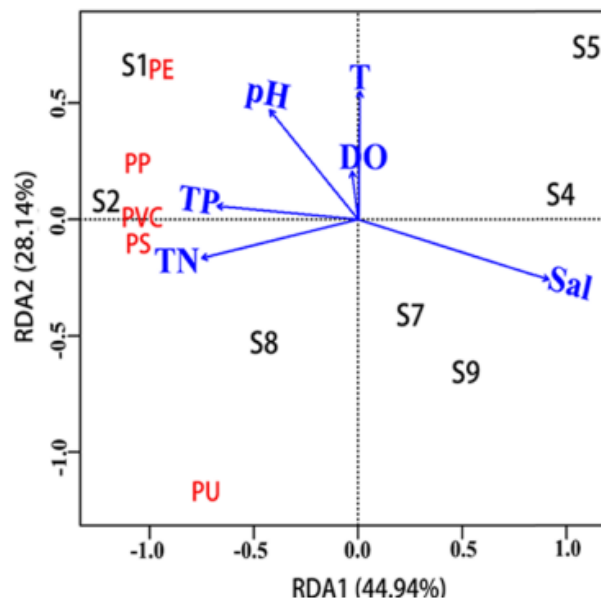
Seeley et al. 2020. Nat. Commun.

What is the impact of microplastic size on the assembly of *in situ* biofilm communities in the ocean?

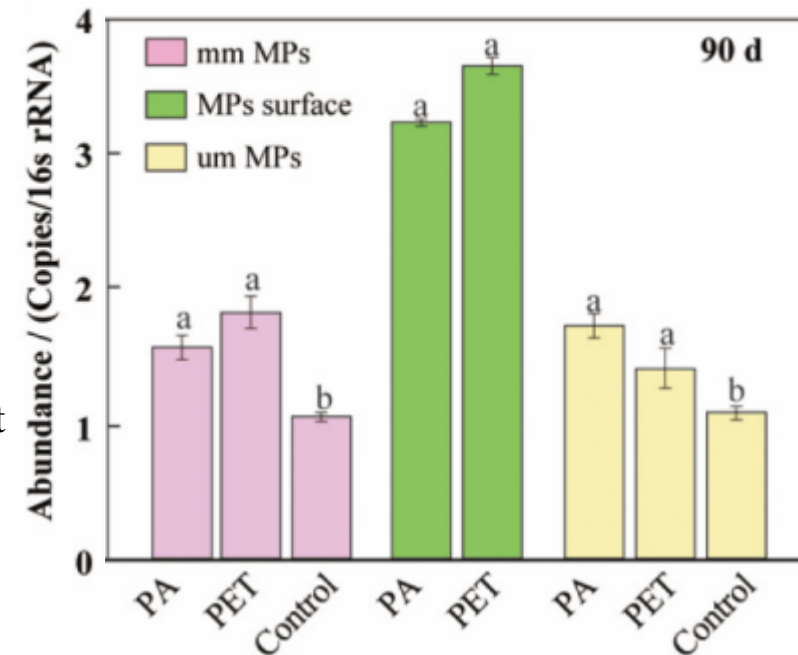
- Multiple factors can affect the community structure of surface biofilms on marine microplastics (MPs);
- Bacteria settlement on MPs surface is affected by factors such as polymer types, exposure time, seawater salinity, plastic color;
- Some studies have focused on the relationship between MPs size and the functional characteristics of biofilm, such as in wastewater treatment systems, where MPs size can affect the environmental behavior of antibiotic resistance genes.



Zhang et al. 2022. J. Hazard. Mater.



Li et al. 2019. Environ. Sci. Technol.



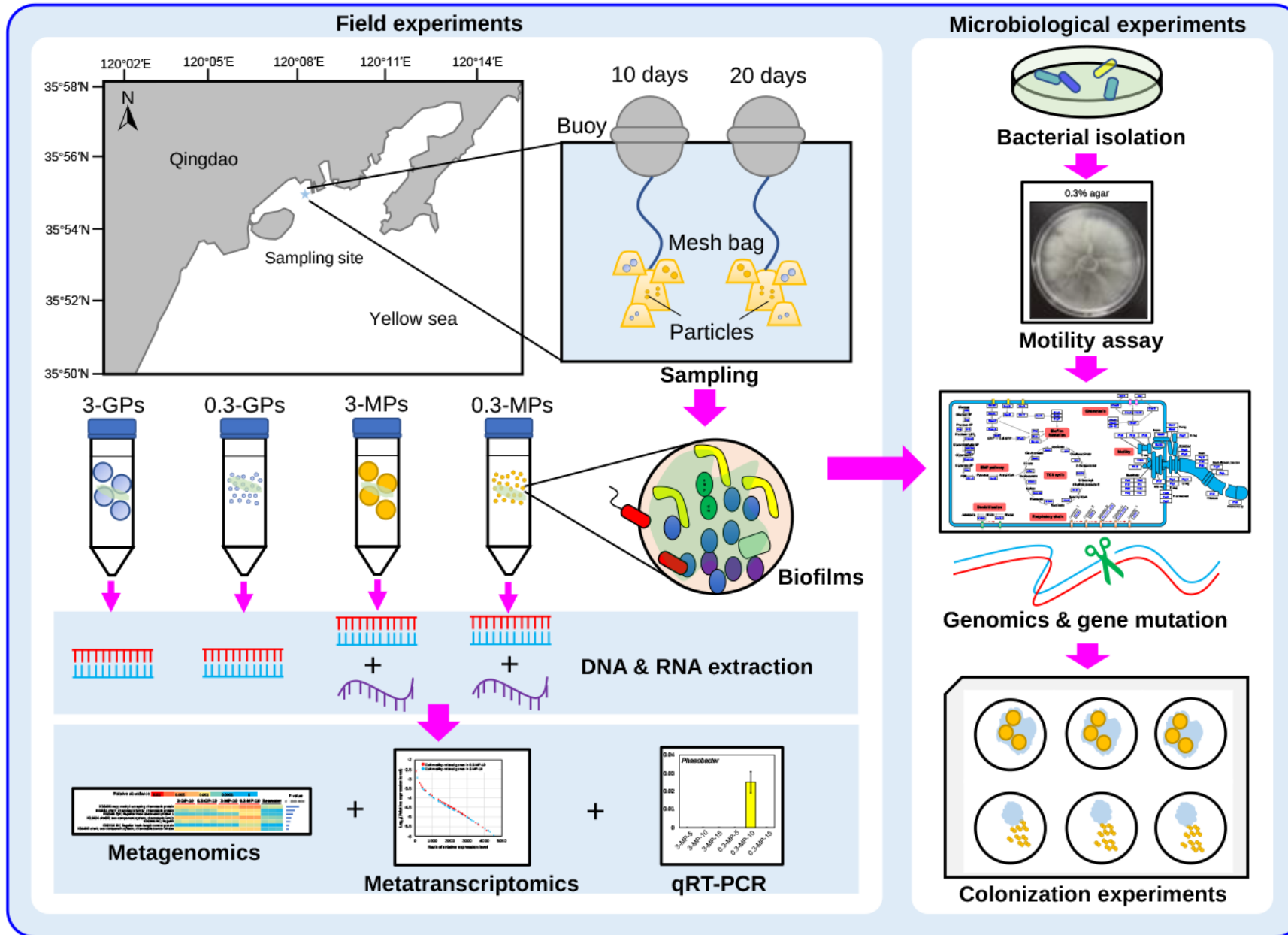
Wang et al. 2022. Sci.Total. Environ.

Scientific Question:

- Does the size of MPs affect the assembly of *in situ* biofilm communities in the ocean, and what are the specific mechanisms involved?



Experimental design

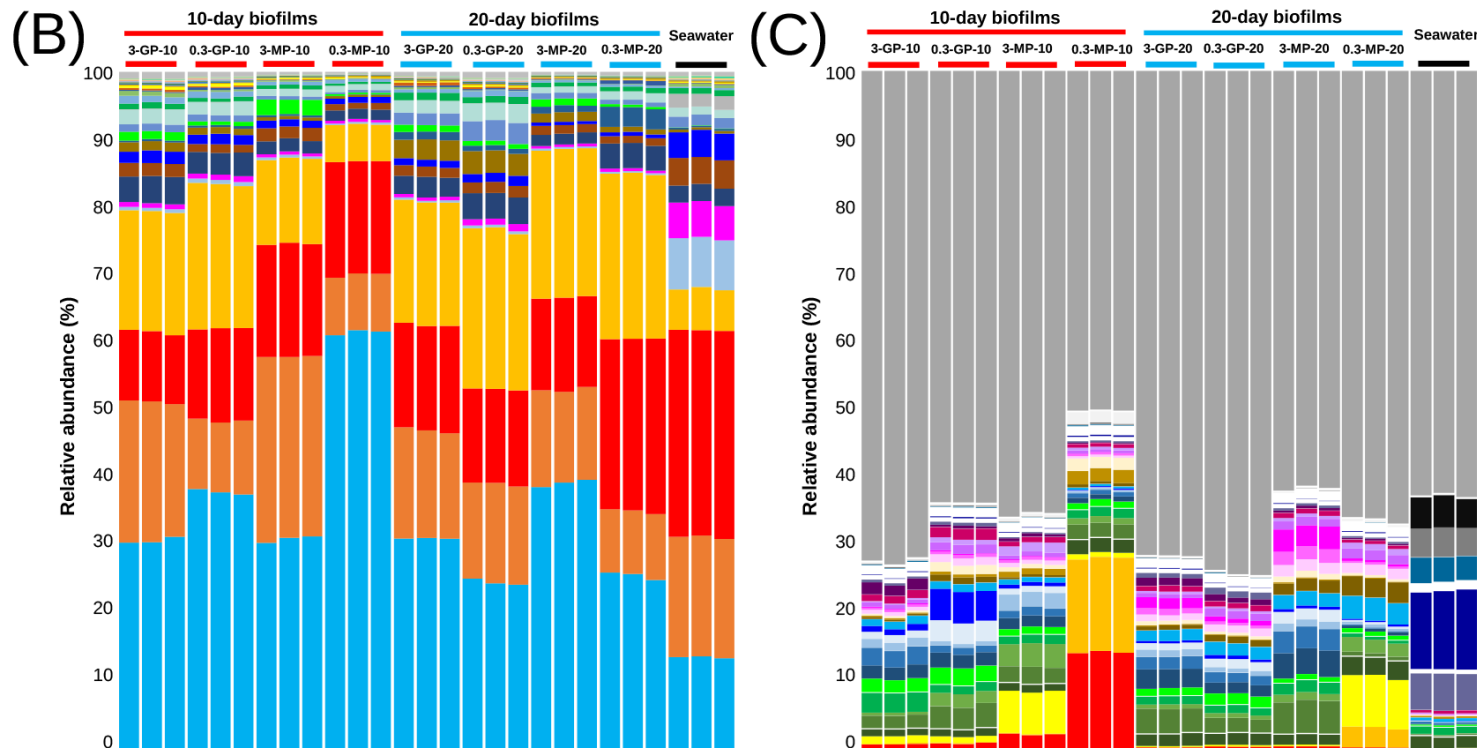
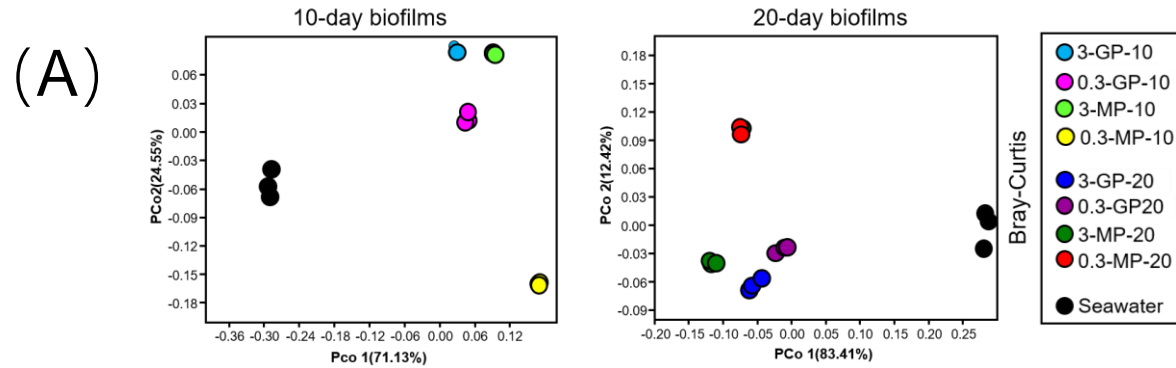


- Place two sizes (3mm and 0.3mm) of microplastics and glass particles in the intertidal zone, and recover the developed biofilm after 10 and 20 days;
- At the community level: metagenomics, 16S rRNA gene amplicon sequencing, metatranscriptomics, and qRT-PCR techniques;
- At the single-bacterium level: physiological and biochemical experiments including bacterial motility assays, MPs colonization assays, bacterial co-culture experiments, and genetic knock-out experiments validation.



Results

Biofilms of MPs and GPs, as well as species composition of marine microbial community in seawater.



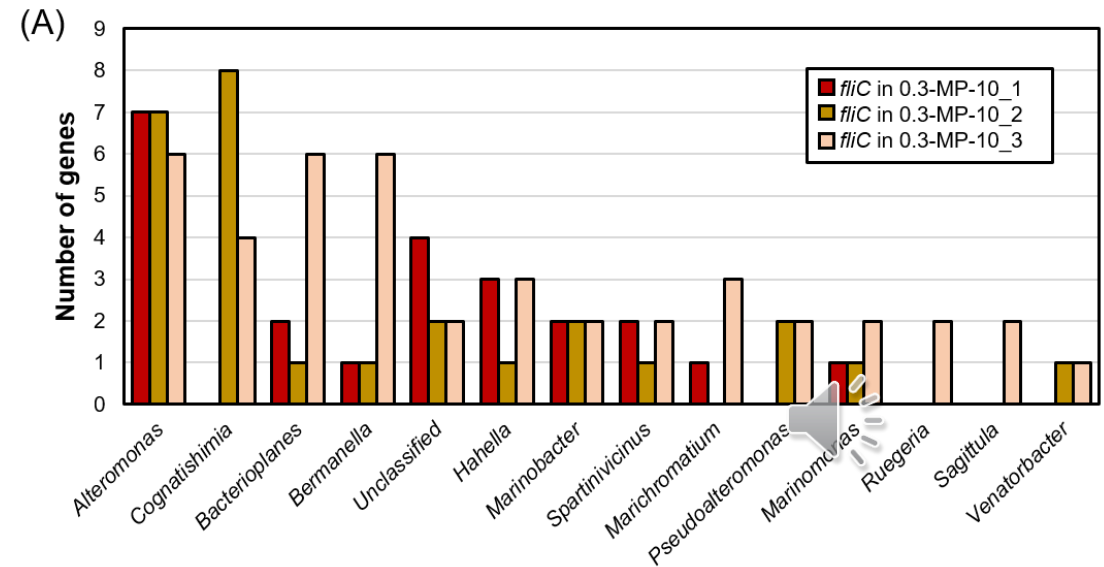
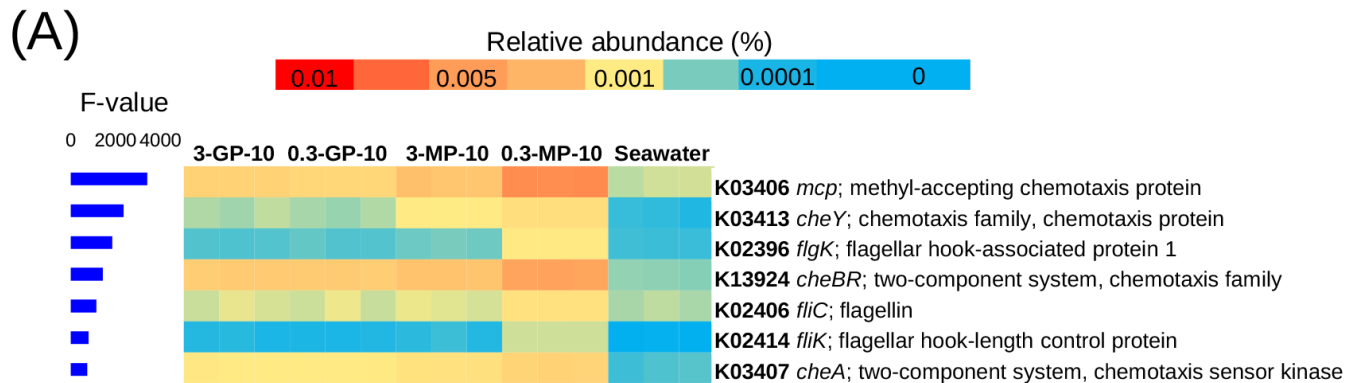
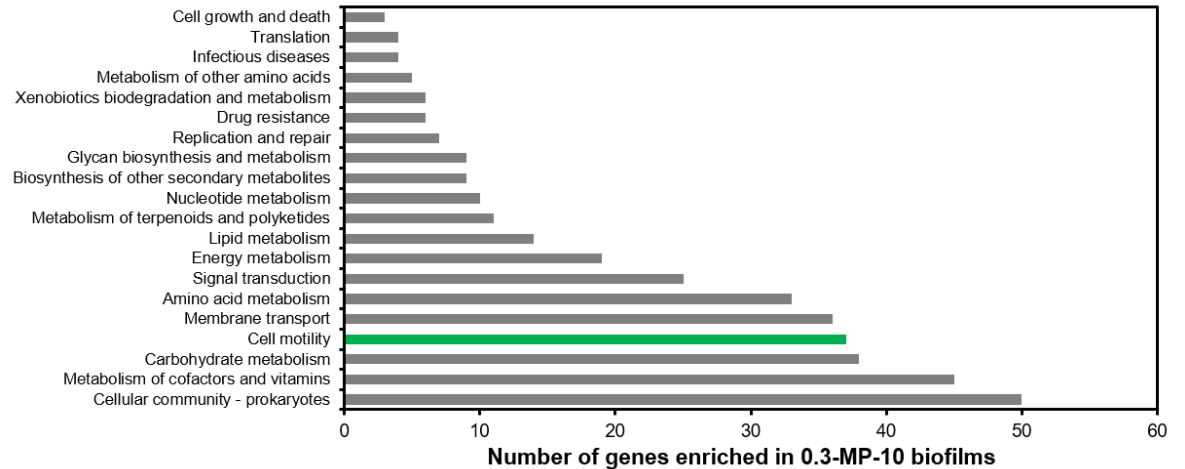
- Species composition of marine microbial community in seawater is different from all biological biofilm samples;
- The community structure of 0.3-MP-10 is relatively unique, with the highest abundance of γ -Proteobacteria, and *Oleiphilus* and *Oleibacter* as the main dominant genera. The species composition of biological biofilm communities at different development times undergoes significant structural changes;
- In PCoA, there is a significant difference between the 0.3-MP-10 and other biofilm communities.



Results

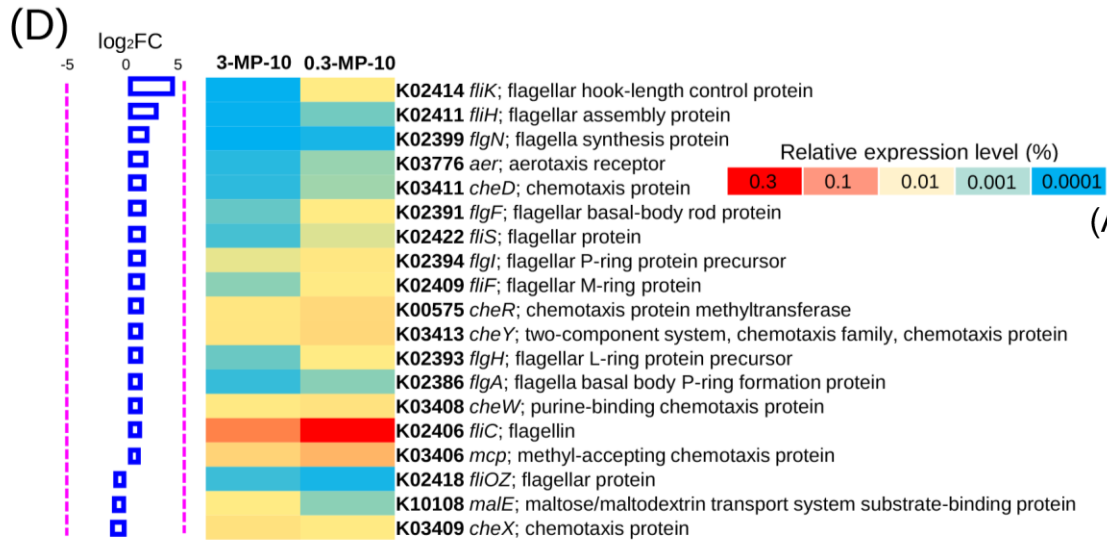
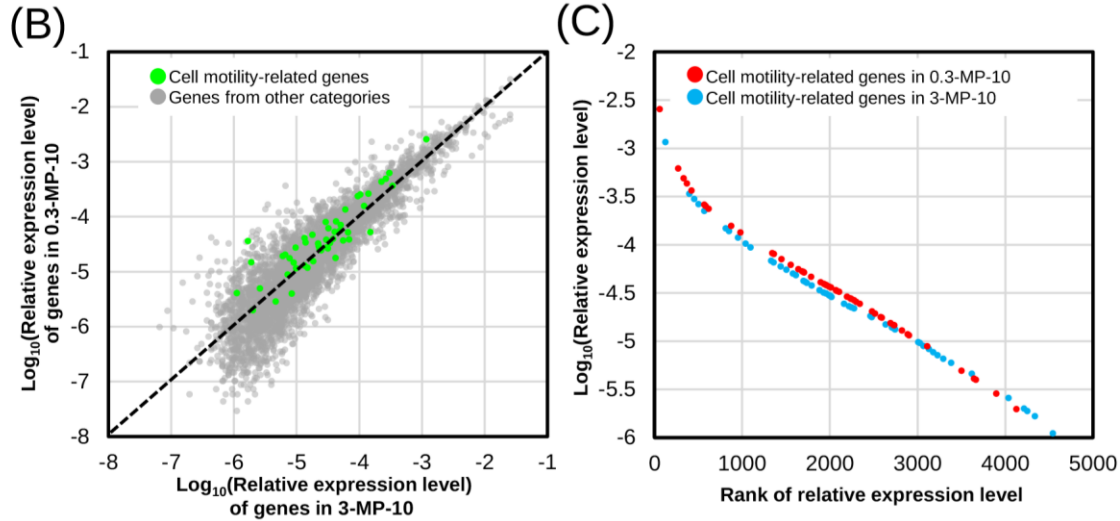
Genes related to motility and chemotaxis are enriched in the biofilm community of 0.3-MP-10.

- One-way ANOVA: Motility genes are one of the significantly enriched gene categories in 0.3-MP-10, with 7 motility-related genes in the TOP40;
- DESeq2: Almost all motility-related genes show significant differential expression and enrichment in 0.3-MP-10;
- *fliC* and *flgE* are mainly present in the biofilm communities of *Alteromonas*, *Cognatishimia*, and some members of Roseobacter group in 0.3-MP-10.

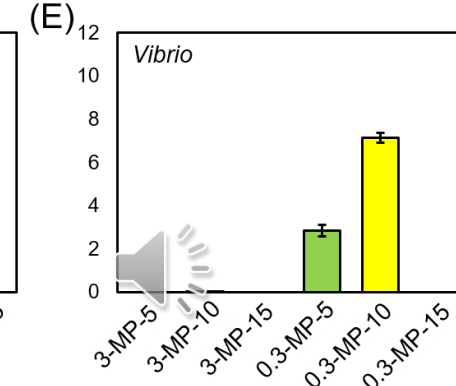
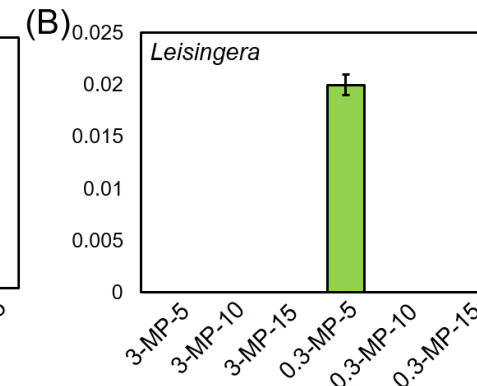
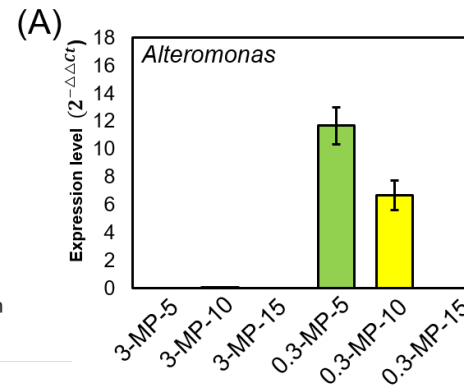


Results

Enrichment and *in situ* expression of motility and chemotaxis genes in 0.3-MP-10 Biofilm.



- The relative expression levels of motility-related genes in the 0.3-MPs-10 biofilm community are higher than those in the 3-MPs-10 community, such as *fliK* and *flgF*;
- Active *fliC* and *flgE* are mainly present in the *Alteromonas*, *Pseudoalteromonas*, and *Thalassolitus* in the 0.3-MPs biofilm community;
- qRT-PCR analysis showed that the expression level of *flgE* in the 0.3-MPs biofilm community was significantly higher than that in the 3-MPs community, and it was only expressed in the early stages (5 or 10 days).

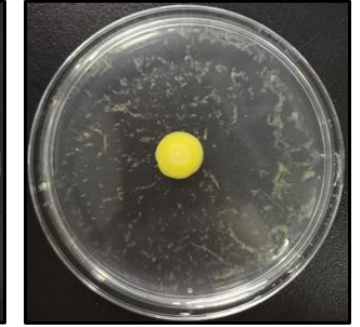
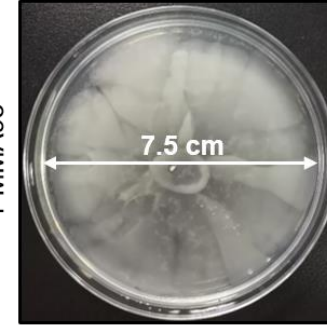


Results

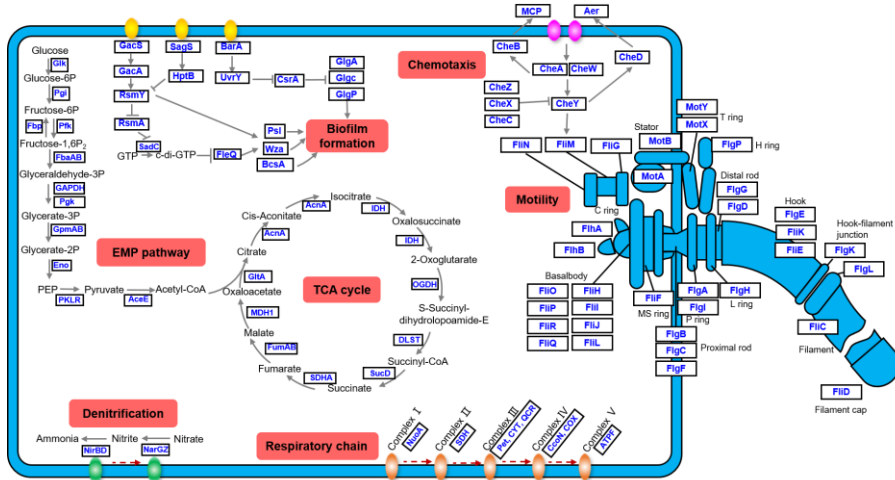
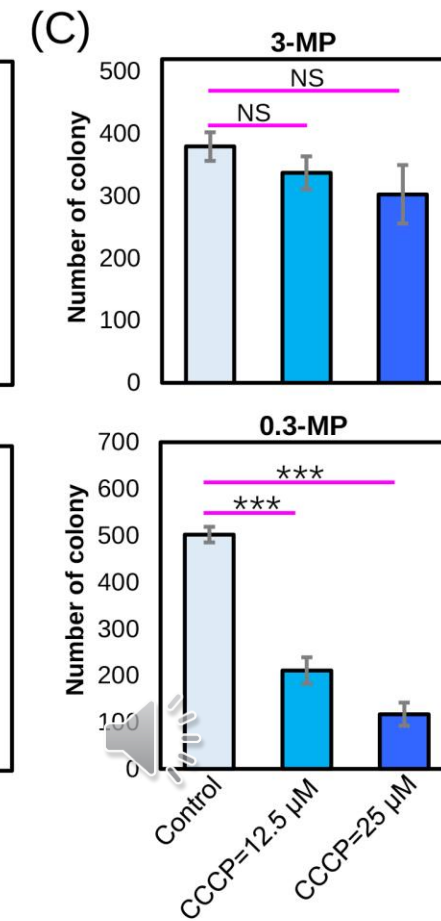
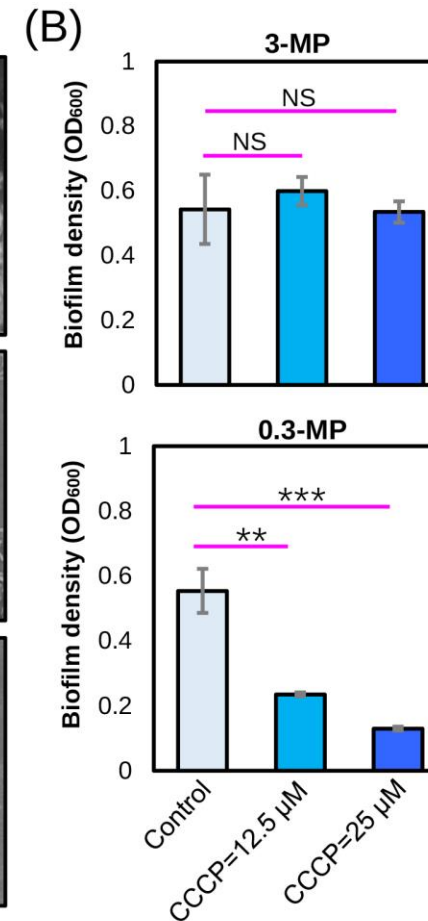
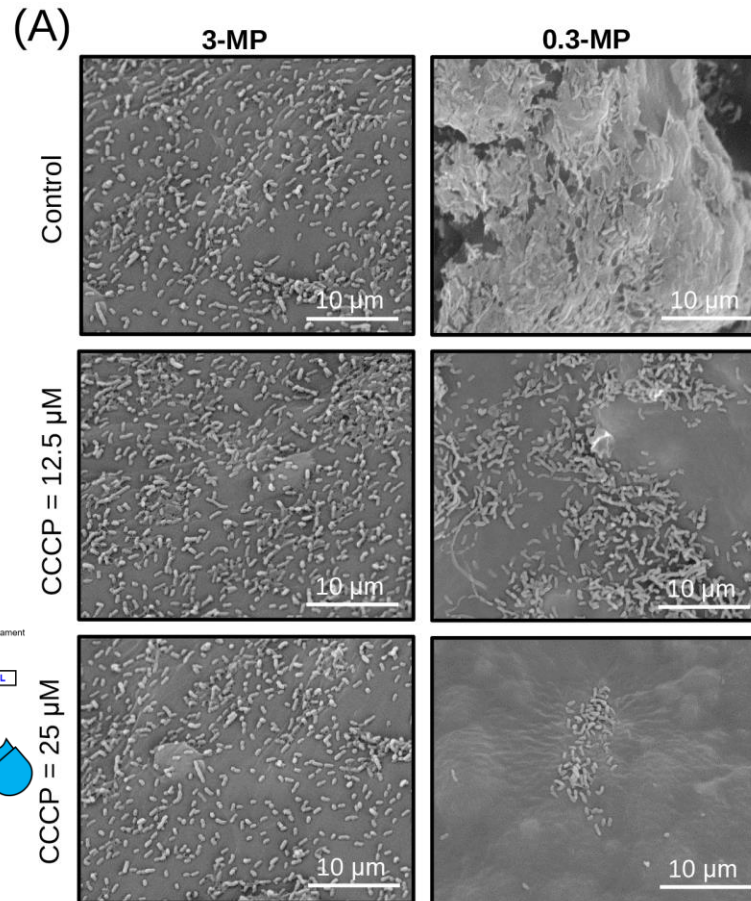
The motility of bacteria is strongly correlated with their colonization on 0.3-MPs.

- A bacterial strain belonging to the *Alteromonas* (PMMA93) was selected from the biofilm of MPs, and single-cell experiments were conducted. Whole genome sequencing analysis showed that it has a relatively complete pathway for the biosynthesis of flagella;
- The motility experiment shows that it has strong swimming and swarming ability;
- The MPs settlement experiment showed that the settlement of PMMA93 on 0.3 mm microplastics was related to bacterial movement driven by proton motive force.

Alteromonas sp009811495
PMMA93

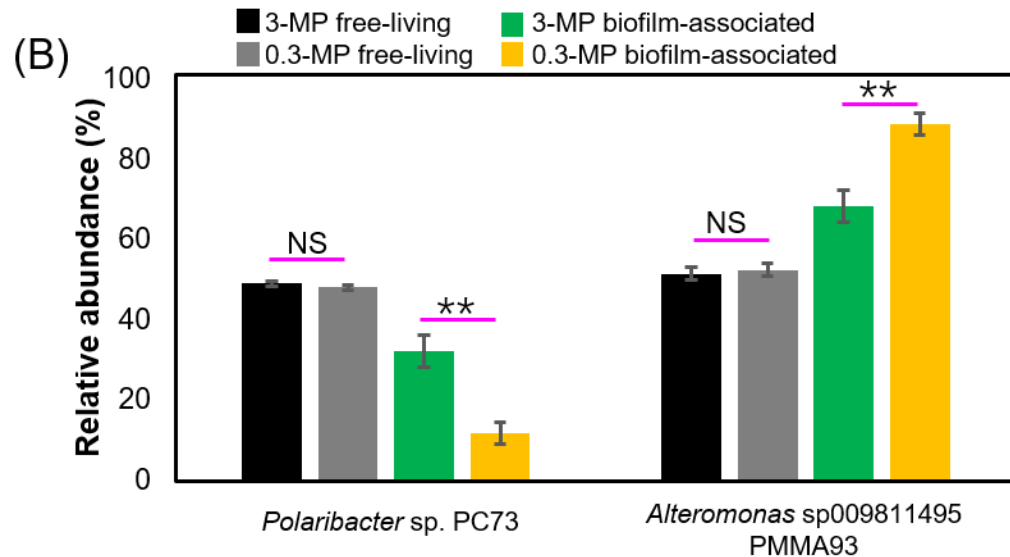
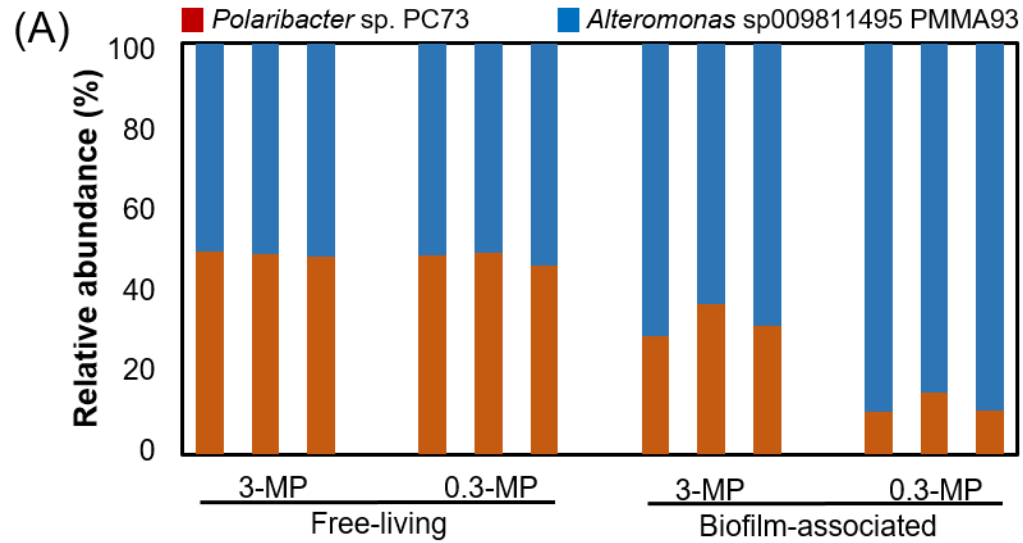


Polaribacter dokdonensis
PC73

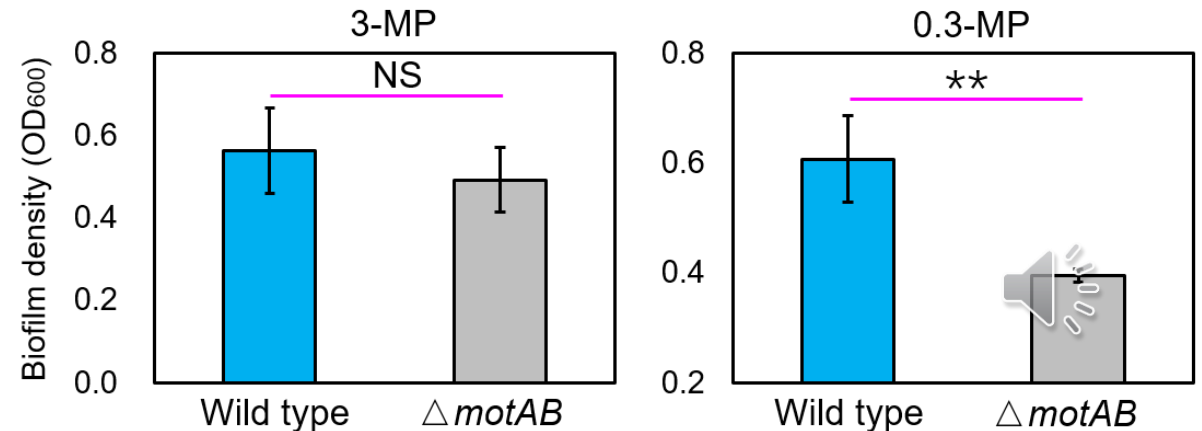


Results

The motility of bacteria is strongly correlated with their settlement on 0.3-microplastics.



- The MPs settlement experiments of the other three bacterial genera isolated from the surface of MPs were similar to those of PMMA93, indicating that the motility of strains promoting their settlement on 0.3-MPs is universal;
- Co-culture settlement experiments of motile and non-motile bacteria showed that motility enhanced the settlement ability of bacteria on 0.3-microplastics;
- The *Vibrio* mutant strain with knocked-out flagellar genes significantly reduced its settlement on 0.3-microplastics.

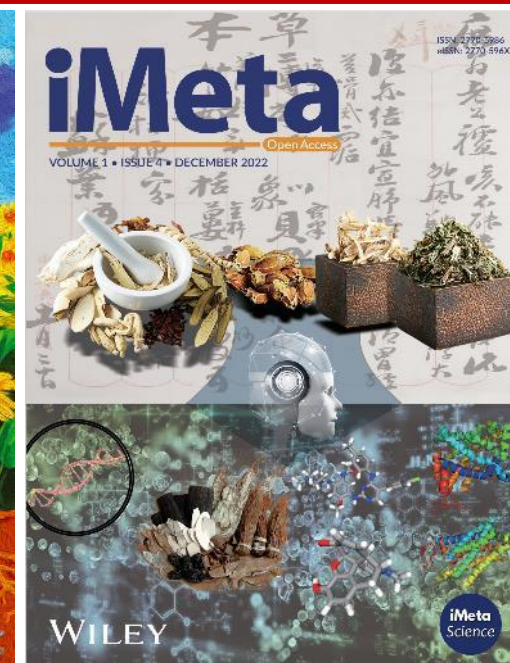


Conclusions

- ◆ The community structure of the biofilm on the surface of 0.3 mm microplastics is significantly different from that of all other biofilms (including 3 mm microplastics and 0.3&3 mm glass).
- ◆ Compared to the 3 mm microplastics, the biofilm on the surface of 0.3 mm microplastics is enriched with genes involved in flagellar motility and chemotaxis, indicating that the community is more "motile".
- ◆ Laboratory experiments with isolated bacterial strains confirmed a strong correlation between bacterial motility and their colonization on 0.3 mm microplastics.



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