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Published in:
FEMS Microbiology Letters

Link to article, DOI:
10.1093/femsle/fnab026

Publication date:
2021

Document Version
Peer reviewed version

Effect of polymer type on the colonization of plastic pellets by marine bacteria

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Keywords: Marine bacteria; microbiome; plastic; microbial colonization; plastisphere; biofilm

ABSTRACT: Plastic is omnipresent in the oceans and serves as surface for biofilm-forming microorganisms. Plastic debris comprises different polymers, which may influence microbial colonization and here, we evaluated if polymer type affects bacterial biofilm formation. Quantifying the biofilm on polyethylene (PE), polypropylene (PP) or polystyrene (PS) pellets by six marine bacterial strains (*Vibrio, Pseudoalteromonas, Phaeobacter*) demonstrated that each strain had a unique colonization behavior with either a preference for PS or PP over the other polymer types or no preference for a specific plastic type. PE, PP, and PS pellets were exposed to natural seawater microbiota using free-living or total communities as inoculum. Microbial assembly as determined by 16S rRNA (V4) amplicon sequencing was affected by the composition of the initial inoculum and also by the plastic type. Known polymer and hydrocarbon degraders such as *Paraglaciecola*, *Oleibacter*, and *Hydrogenophaga* were found in the plastic biofilms. Thus, on a community level, bacterial colonization on plastic is influenced by the microorganisms as well as the polymer type, and also individual strains can demonstrate polymer-specific colonization.

INTRODUCTION

Over the past eighty years, plastic production has been growing continuously resulting in an estimated 8 – 13 million metric tons of plastic waste annually entering the oceans (Cozar *et al.* 2014; Jambeck *et al.* 2015; van Sebille *et al.* 2015; Cózar *et al.* 2017; Barrows, Cathey and Petersen 2018; Peeken *et al.* 2018). Plastic debris comprises different polymer types and shapes (Hidalgo-Ruz *et al.* 2012) and is continuously exposed to and may be modified by abiotic forces and mechanical actions (Andrady 2017). Additionally, living organisms interact with the

material and specifically microorganisms have been proposed to affect the fate of plastic in the marine environment (Cozar *et al.* 2014; Rummel *et al.* 2017). Recent studies have demonstrated that plastic particles of all sizes, including microplastics (< 5 mm), are readily colonized by different groups of marine microorganisms, notably bacteria (Zettler, Mincer and Amaral-Zettler 2013; Oberbeckmann *et al.* 2014; De Tender *et al.* 2015; Bryant *et al.* 2016; Debroas, Mone and Ter Halle 2017; Dussud *et al.* 2018b; Ogonowski *et al.* 2018; Amaral-Zettler, Zettler and Mincer 2020; Oberbeckmann and Labrenz 2020). Within days, microbial biofilms are already formed on virgin plastic (Dang *et al.* 2008; Lobelle and Cunliffe 2011; Oberbeckmann, Kreikemeyer and Labrenz 2018; Kesy *et al.* 2019). Colonization of plastic surfaces may benefit the bacteria allowing spatial transportation or genetic exchange (Kooi *et al.* 2017; Arias-Andres *et al.* 2018). Also, some strains may be able to degrade this presumed inert material or its additives and use it as carbon source (Roager and Sonnenschein 2019). Many studies found that the microbial composition on plastic, the 'plastisphere', is unique in comparison to that of the surrounding seawater and to those of other materials or marine particles such as plankton (Zettler, Mincer and Amaral-Zettler 2013; De Tender *et al.* 2015; Bryant *et al.* 2016; Debroas, Mone and Ter Halle 2017; Dussud *et al.* 2018b; Ogonowski *et al.* 2018), however, others did not identify a difference between biofilms on plastic and on non-plastic materials (wood, glass) or only for a sub-group of bacteria (Oberbeckmann, Osborn and Duhaime 2016; Oberbeckmann, Kreikemeyer and Labrenz 2018). Colonizing plastic is assumed to be a prerequisite for any possible degradation (Tribedi and Sil 2013) and surface properties may affect colonization. Since plastic comprises different polymer types such as polyethylene, polypropylene and polystyrene (Koelmans, Besseling and Shim 2015), a polymer-specific colonization by bacteria could suggest the active utilization of the surface as carbon source (Zettler, Mincer and Amaral-Zettler 2013; Ogonowski *et al.* 2018).

However, studies focusing on the marine microbial diversity on different plastic polymers have not reached the same conclusions in terms of if and how the polymer types affect community composition (Oberbeckmann, Kreikemeyer and Labrenz 2018; Ogonowski *et al.* 2018; Kesy *et al.* 2019). Pinto *et al.* found that polymer type only influences a part of the biofilm community (Pinto *et al.* 2019) indicating that individual bacterial strains contribute to a polymer-specific diversification. However, we currently have no knowledge on how individual marine strains colonize different polymer types. The purpose of the present study was to determine if plastic type affected the colonization behavior of individual bacterial strains and microbial communities on three plastic polymer types. We selected 5 mm plastic pellets that are a common type of marine plastic waste (Koelmans, Besseling and Shim 2015) and are an easy-to-handle plastic form for laboratory and *in situ* experiments (Oberbeckmann, Kreikemeyer and Labrenz 2018; Ogonowski *et al.* 2018).

MATERIALS AND METHODS

Colonization of PE, PS and PP pellets by individual bacterial strains. Six marine bacterial strains isolated from Danish coastal waters were previously identified as good biofilm formers on plastic (Bernbom *et al.* 2011) and used in this study: *Pseudoalteromonas piscicida* B39bio, *Pseudoalteromonas ulvae* H34q-5a and A24a-4a, *Vibrio* sp. A31bio, *Phaeobacter* sp. A36a-5a and A49a-4a (Bernbom *et al.* 2011). The strains were cultivated from freeze stock on marine agar (MA; Difco 2216) at 25°C. Pre-cultures were grown overnight in 10 ml 2% sea salts (SS) (Sigma-Aldrich) with 0.3% Bacto casamino acids (BD) in 50 ml tubes at 25°C and 200 rpm. The pre-cultures were harvested (1 min at 11,337 x g) and washed twice to remove any excess

nutrients. The pellet was resuspended in 2% SS and diluted to an optical density (at 600 nm) of 0.01 in 2% SS. Five mL of diluted culture was incubated with five polyethylene (PE; Purell, PE 1840H), polypropylene (PP; SABIC, PP 579S), or polystyrene (PS; Polystyrol 454H) pellets (sterilized for 15 mins in 70% ethanol) in glass tubes at 25°C and 200 rpm for 24 hours. The pellets had a diameter of ~ 5 mm and the shape differed slightly between polymer types, but pellets of the same plastic type were very similar. After incubation, the colony-forming units in the suspension and on the pellets were assessed by tenfold dilutions in 2% SS and plating on MA. To count surface attached bacteria, the plastic pellets were washed twice with 2% SS and sonicated in a 28-kHz sonication bath (Delta 220T; Aerosec Industrie) for 2 mins to remove the biofilm cells from the pellet surface. The experiment was performed in biological duplicates.

Colonization of PE, PS and PP pellets by natural marine communities. To test if polymer type affected the microbial diversity on plastic, the free-living fraction of seawater (< 5 µm) or total seawater was incubated with PE, PS and PP pellets and the diversity of the plastic biofilm was analysed after two weeks. The plastic biofilm was compared to the initial seawater microbiota and seawater incubated without plastic for two weeks (Table S1). Natural seawater was sampled on February 2, 2017, in Hellerup harbour (55.732103 N, 12.580680 E). The seawater temperature was 0.6°C, salinity 9.8 PSU and dissolved oxygen concentration was 4.7 mg/L as measured using a handheld Professional Plus instrument (YS6050000; YSI, Yellow Springs, OH). To obtain the initial microbial diversity of the seawater (free-living and total seawater inoculum), 200 mL seawater were filtered through 5 µm polycarbonate (PC) filters (GE Water & Process Technologies, K50BP02500) supported by an 8 µm methylcellulose (MCE) filter (25 mm, GE Water & Process Technologies, E80WP02500)and subsequently 0.2 µm PC filters (25 mm, Anodisc 25, WhatmanTH, 6809-6009) supported by a 1 µm MCE filter (25 mm,

ADVANTEC, A100A025A) in triplicates and stored at -20°C until DNA extraction (for subsequent amplicon sequencing).

Fifty milliliters of total seawater or 5 µm-filtered seawater (free-living fraction) were incubated in 250 mL Schott flasks with 4.3 g of either PE, PP, or PS pellets (sterilized with 70% ethanol). Controls of 50 mL total seawater and 5 µm-filtered seawater with no pellets added were included. Triplicates of each combination were incubated for two weeks at 25°C and 150 rpm. Samples including the pellets were poured into 50 mL falcon tubes and liquid was removed by pipetting. Forty milliliters of the control samples without plastic pellets was filtered onto a 0.2 µm PC filters. Plastic pellets and filters were stored at -20°C until DNA extraction.

DNA extraction and sequencing DNA extraction of the initial seawater community, the plastic biofilms and seawater controls (after 2 weeks of incubation) was carried out after modified protocols by Rygaard *et al.* (Rygaard *et al.* 2017) and Boström *et al.* (Boström *et al.* 2004). Filters and plastic pellets were incubated in 3.2 mL lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.73 M sucrose) and 0.5 mg/mL lysozyme (Sigma, L6876) for 30 min at 37°C and 150 rpm. 37.2 mg acid-washed glass beads (Sigma-Aldrich, G1145, 150 – 212 mm) were added and samples were vortexed for 1 min at 2000 rpm. 0.5 mg/mL Proteinase K (VWR Life Science, E195) and 1% sodium dodecyl sulfate were added and samples were incubated over night at 55°C and 150 rpm. The lysate was transferred to new tubes without transferring plastic pellets, filters or glass beads. Pellets or filters were washed once with 400 µL TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8) and the washing solution was added to the lysate. The mixture was extracted with phenol: chloroform:isoamyl alcohol (25:24:1 vol/vol/vol) and subsequently, with chloroform:isoamyl alcohol (24:1 vol/vol). The DNA was precipitated with ethanol and sodium acetate at a final concentration of 75% and 0.5 M, respectively, for 2 hours at -20°C.

After centrifugation at 20,000 x g and 4°C for 30 min, DNA pellets were washed with ethanol and dried before dissolving in MilliQ water. DNA quantity and quality was assessed by Denovix DS-11 (DeNovix Inc.) and Qubit® 2.0 Fluorometer (Invitrogen).

The V4 region of the 16S rRNA gene was amplified using primer pair 515FB-806RB (Apprill *et al.* 2015; Parada, Needham and Fuhrman 2016) with sequencing-specific overhangs (forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG- GTGYCAGCMGCCGCGGTAA-3'; reverse: 5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GGACTACNVGGGTWTCTAAT-3') using 2x KAPA HiFi HotStart ReadyMix (Roche). The procedure followed the 16S Illumina Amplicon Protocol by the Earth Microbiome Project (https://earthmicrobiome.org/protocols-and-standards/16s/) with the following adaptations of the PCR settings according to the polymerase used herein: 95ºC for 3 mins, 25 cycles of 98ºC for 20 secs, 53ºC for 15 secs, 72ºC for 15 secs with a final extension at 72ºC for 1 min. After PCR cleanup using AMPure XP beads (Beckman Coulter), a dual-indexing PCR was conducted to generate a barcoded Illumina MiSeq library using 2x KAPA HiFi HotStart ReadyMix (Roche) and the Nextera XT v2 indexing kit (Illumina) following the manufacturer's guidelines. The indexed amplicons were again purified using AMPure XP beads (Beckman Coulter). DNA quantity and quality was assessed on a Qubit® 2.0 Fluorometer and a Bioanalyzer DNA 1000 chip (Agilent Technologies). The libraries were normalized to 4 nM before pooling of the samples and addition of the PhiX control (5%). Sequencing of paired-end reads was carried out on an Illumina MiSeq Personal Sequencer using a MiSeq v2 2x250 sequencing kit (Illumina) at the Novo Nordisk Foundation Center for Biosustainability resulting in 17 mio identified reads. All raw sequence files are available from the NCBI Short Read Archive (SRA) database (BioProject: PRJNA484726; BioSamples: SAMN09768739-68). Before further analysis, the

sequences of the samples of the particle-attached seawater fraction (> 5 µm) were merged with the corresponding samples of the free-living fraction (0.2 - 5 µm) to obtain the sequences of the total initial seawater communities, i.e. samples 25, 26, and 27 (Table S1).

Analysis of amplicon sequences. The QIIME 2 pipeline (Bolyen *et al.* 2019) was used via docker (core:2017.12) for read and diversity analysis including statistics applying DADA2 (Callahan *et al.* 2016) for quality control and feature table construction following the moving pictures tutorial (version 2017.12). Taxonomic classification was performed against the SILVA database v132 (trained for the 515-806 region of the 16S rRNA gene using the taxonomy according to 99% identity) (Quast *et al.* 2013). To assess from which initial inoculum (free-living or total seawater) the plastic biofilm communities originated, Venn diagrams were build using Venny 2.1 (Oliveros 2015) comparing the OTUs of each plastic biofilm to those of the initial free-living seawater fraction and those of the initial total seawater used as inocula. Abundances of specific operational taxonomic units (OTUs) were log10(x+1) transformed to normalize the data and a heatmap was made using the pheatmap package v1.0.12 (Kolde 2019) in R. Differential abundances were tested using ANCOM (Mandal *et al.* 2015). Most abundant OTUs were verified by BLASTn (Altschul *et al.* 1990) against the 16S rRNA gene database. Multivariate analysis was conducted using non-metric multidimensional scaling on Bray-Curtis distances using phyloseq v1.30.0 (McMurdie and Holmes 2013) and ggplot2 v3.3.2 (Wickham 2016) in R. PERMANOVA using the adonis function from the vegan package v2.5-6 (Oksanen *et al.* 2019) on the Bray-Curtis distances was applied to test the significance of incubation with different plastic types for each seawater type separately (999 permutations). Differences in community composition between those sample types were assessed with homogeneity of dispersions tests on Bray-Curtis dissimilarity matrices using the betadisper and permutest

functions (999 permutations) (Anderson 2006) from the vegan package version 2.5-6 (Oksanen *et al.* 2019).

RESULTS AND DISCUSSION

Effect of plastic type on colonization of individual bacterial strains. Six marine bacterial strains were incubated with PE, PS and PP pellets. The level of bacterial colonization varied from 4.6 $\log_{10}$ CFU/pellet ± 0.0 (*P. ulvae* A24a-4a on PE) to 6.8 $\log_{10}$ CFU/pellet ± 0.4 (*Phaeobacter* sp. A36a-5a on PS) (Figure 1A) predominantly reflecting cell numbers in suspension that ranged from 4.5 $\log_{10}$ ± 0.2 (*P. ulvae* A24a-4a with PS) to 6.9 $\log_{10}$ ± 0.0 CFU/mL (*P. piscicida* B39bio with PS) (Figure S1A). Variation in the total cell numbers between strains could be due to the initial calibration to the same optical density value, which might translate into a different CFU for each strain (Figure S1B). The ratio of total plastic pellet CFUs over total suspension CFUs per sample demonstrates that the majority of B39bio cells were planktonic (ratio < 1), while in the most samples of the other five strains, the majority of cells colonized the pellets (ratio > 1, Figure 1B). The ratio also indicates that each strain colonized the three pellet types to different degrees, e.g. *P. ulvae* A24a-4a colonized PS with higher numbers than PP and PE and *P. ulvae* H34q-5a and *Vibrio* sp. A31bio preferred PP over PS and PE. In case of A24a-4a, the effect could be due to the different hydrophobicities of the three substrates with PE being most and PS being least hydrophobic. But also overall hydrophobicity of the cells, their nutritional status and the ratio of intracellular vs. extracellular nutrients or salts (Karatan and Watnick 2009) could affect the ability of the strains to adhere to different surfaces. The cell hydrophobicity is influenced by the membrane composition including

its lipids, membrane proteins and oligosaccharides (Krasowska and Sigler 2014) and would be strain-specific. To the best of our knowledge, so far, no study has investigated which strain-specific characteristics would be responsible for a polymer-specific colonization of plastic, thus we cannot further speculate on the reasons for this differentiated phenotype in case of the herein utilized strains.

Effect of plastic type on colonization of marine bacterial communities. To assess if the polymer type had an effect on the colonization by a natural marine bacterial community, PE, PS, and PP pellets were exposed to natural seawater (pre-filtered on 5 µm, herein referred to as the 'free-living' microbial community as well as unfiltered, 'total' seawater) for two weeks in a laboratory setup and the composition of the microbial biofilm on the pellets was analyzed by sequencing of the V4 region of the 16S rRNA gene. In total, 1.7×10^7 V4 sequences were obtained. The plastic biofilm samples (samples 1-18, Table S1) were compared to the initial free-living (samples 28-30) and total seawater communities (samples 25-27) used as inocula and the free-living (samples 22-24) and total seawater (samples 19-21) controls incubated without addition of pellets. The sequencing depth as determined by rarefaction was sufficient to cover the diversity of the microbiomes (Figure S2). Greatest richness was observed in the initial total seawater community as these sample type comprises all microbiota added into the experiment (Figure S3). There was no statistical difference in the richness in a pairwise comparison of samples ($P > 0.05$, see Table S2 for full data). Highest evenness was detected in the initial total seawater sample, but again, the samples did not differ significantly in a pairwise comparison ($P > 0.05$, Table S2, Figure S4).

The initial total seawater community was dominated by Gammaproteobacteria (21%), Bacteroidia (18%), Alpha- (17%), Deltaproteobacteria (9%) and Actinobacteria (3%) (Figure 2). The dominant classes in the initial free-living seawater community were Alpha- (29%) over Gammaproteobacteria (22%), Bacteroidia (12%) and Actinobacteria (9%). After incubation without plastic, Alphaproteobacteria (40-68%) were dominant in both (total and free-living) seawater communities followed by Gammaproteobacteria (20-52%). The microbial communities recovered from the three plastic types were dominated by Gammaproteobacteria (47-81%) followed by Alphaproteobacteria (7-34%) and Bacteroidia (3-20%) irrespective of the inoculum (free-living fraction or total seawater). Alpha- and Gammaproteobacteria are generally abundant on marine plastic debris across different ocean regions (Zettler, Mincer and Amaral-Zettler 2013; Oberbeckmann *et al.* 2014; De Tender *et al.* 2015; Oberbeckmann, Osborn and Duhaime 2016). These groups were also found to colonize virgin plastic pellets after one week of incubation with Baltic seawater and were significantly more abundant in plastic biofilms than in the seawater inoculum (Kesy *et al.* 2019). While Ogonowski *et al.* also identified Alphaproteobacteria as dominant in plastic biofilms after a two-week incubation of Baltic seawater (salinity of 3.5) with plastic beads, Gammaproteobacteria were rare, also in the inoculum, possibly due to the lower salinity in sampled seawater (Ogonowski *et al.* 2018). Indeed salinity was identified as one of the parameters contributing to shaping the plastic biofilm diversity, particularly enhancing the plastic biofilm abundance of Gammaproteobacteria (De Tender *et al.* 2015; Oberbeckmann, Kreikemeyer and Labrenz 2018; Kesy *et al.* 2019). Thus, the biofilm communities assembled herein derived from a seawater community with a salinity of 9.8 are in a higher-taxa comparison similar to other marine communities on plastic debris.

In the Bray–Curtis dissimilarity analysis, replicates of the same sample type (as defined by the inoculum type (free-living or total seawater), incubation time (0 or 2 weeks), and type of pellets added (PE, PP, or PS)) grouped together (Figure 3). The free-living and total seawater microbial communities incubated without plastic pellets changed similarly during the two week incubation. The plastic microbial biofilm communities differed from the initial seawater communities and the seawater control without plastic. The water type of the inoculum (free-living fraction or total seawater) significantly affected the composition of the microbial biofilm communities on plastic (PERMANOVA on all plastic biofilms from free-living fraction or total seawater: $R^2 = 0.40635$, $P = 0.001$; Table S3); however, PERMDISP comparison indicates dispersion effects (Table S3). To assess if free-living or particle-attached bacteria of the inoculum constitute the communities on plastic, the OTUs identified in the initial free-living fraction and those of the initial total seawater were compared to the OTUs of the six different plastic biofilms using venn diagrams (Figure S5). For all plastic types incubated with total seawater (Figure S5 A, C, E), more OTUs appear to have originated from the particle-attached than the free-living fraction of the inoculum (1,161 vs. 578 on PE, 534 vs. 331 on PP, 905 vs. 346 on PS); however, the total number of OTUs was also higher in the attached fraction than the free-living fraction of the initial seawater (13,177 vs. 3,975). While previous studies compared biofilms on plastic incubated in total seawater to the initial free-living and total seawater community (Dussud *et al.* 2018b; Oberbeckmann, Kreikemeyer and Labrenz 2018; Kesy *et al.* 2019), our experiments using the free-living fraction or total seawater as inoculum confirm that plastic biofilms resemble particle-attached rather than the free-living fraction of the seawater inoculum.

When comparing the microbial communities of the three different plastic types (PE, PP, PS) for both inocula separately, they were significantly different (PERMANOVA on plastic biofilms

from total seawater: $R^2 = 0.53656$, $P = 0.003$; plastic biofilms from the free-living fraction of seawater: $R^2 = 0.47857$; $P = 0.003$) (Table S3) and the effect was not caused by dispersion ($P > 0.05$, Table S3) indicating that the plastic type indeed affected community assembly. While there is a consensus across plastisphere studies that the microbial communities on plastic are different to those of the surrounding seawater (Zettler, Mincer and Amaral-Zettler 2013; Oberbeckmann *et al.* 2014; De Tender *et al.* 2015; Bryant *et al.* 2016; Debroas, Mone and Ter Halle 2017; Dussud *et al.* 2018b; Ogonowski *et al.* 2018; Kesy *et al.* 2019; Oberbeckmann and Labrenz 2020), it is study-dependent if an effect of the polymer type on the biofilm assembly on plastic was observed (Oberbeckmann and Labrenz 2020). Studies analyzing the plastisphere have been conducted using various methodologies making it nearly impossible to compare different results and find a potentially universal answer to this open question (Roager and Sonnenschein 2019; Oberbeckmann and Labrenz 2020). Herein, also, OTU sharedness of the PE, PP, and PS biofilms and the inoculum for each water type confirms that each plastic biofilm harbors polymer-specific OTUs (Figure S6). This result agrees with previous studies proposing marine plastic debris as new niche selecting for a unique subset of colonizing organisms (Oberbeckmann *et al.* 2014; Dussud *et al.* 2018b; Kesy *et al.* 2019).

Typical free-living marine bacteria such as those of the SAR11 clade dominated the seawater samples (Figure 4) and the most abundant OTU was identified as an uncultured marine archaeon of the Candidatus genus *Nitrosopumilus*. On PE, PP and PS incubated with free-living bacteria, the most abundant OTU belonged to the family Alteromonadaceae (13-25%). This OTU increased 238-fold in comparison to its relative abundance in the initial free-living microbial community in the inoculum. It was also abundant (2-4%) in the biofilm on all pellets incubated with total seawater with a 15-fold increase from the initial total microbial community. Using

BLASTn, the sequence had 100% to the 16S rRNA gene of *Paraglaciecola hydrolytica* strain S66 isolated from eelgrass (Table S4). *P. hydrolytica* can degrade seaweed polysaccharides including laminarin, porphyran, and carrageenans, and use them as sole carbon source (Bech *et al.* 2017; Schultz-Johansen *et al.* 2018). Another species, *Paraglaciecola agarilytica*, can produce a styrene monooxygenase active on styrene (Pu *et al.* 2018). The most abundant OTUs in the biofilm communities originating from total seawater belonged to the genera *Oleibacter* (PE, 15%) and *Hydrogenophaga* (PP and PS, both 4%). In the initial total seawater, these OTUs had only a relative abundance of 0.04 and 0.26%, respectively. As indicate by its name, *Oleibacter* and its close relatives are known hydrocarbon degraders and have been isolated after oil enrichment from seawater (Yakimov *et al.* 2004; Teramoto *et al.* 2009, 2011; Liu, Bacosa and Liu 2017). Furthermore, they were found as first colonizers of PVC plates that were however quickly overtaken by other species (Pollet *et al.* 2018). Its class of Oceanospirillales was also found enriched in the plastisphere of microplastics (Debroas, Mone and Ter Halle 2017). *Hydrogenophaga* was previously found enriched on plastic compared to non-plastic substrates after two weeks of enrichment with seawater (Ogonowski *et al.* 2018). In a groundwater bioreactor, *Hydrogenophaga* was enriched on gasoline (Daghio *et al.* 2015). Its family of Comamonadaceae includes a number of genera with bioremediation activities such as *Comamonas*, *Delftia*, *Acidovorax*, or *Variovorax* (Nakajima□Kambe *et al.* 1995; Singleton, Ramirez and Aitken 2009; Peixoto, Silva and Krüger 2017; Posman, DeRito and Madsen 2017; Liu *et al.* 2018) and also includes the genus of the PET-degrading *Ideonella* (Yoshida *et al.* 2016). Thus, in accordance with other studies, we observed putative hydrocarbonoclastic bacteria enriched on plastic in comparison to seawater (Zettler, Mincer and Amaral-Zettler 2013; Oberbeckmann, Osborn and Duhaime 2016; Dussud *et al.* 2018a). In contrast to the analysed *P.*

ulvae A24a-4a, the most abundant *Pseudoalteromonas* strain in the total seawater setup appeared to have a preference for PP (12% of community) over PS and PE (on average, 0.4 ± 0.1 and 0.3 ± 0.2%, respectively).

A study by Ogonowski and co-workers observed a differential colonization of marine microbial communities on different polymer types after two weeks of laboratory incubation (Ogonowski *et al.* 2018), while a comparison of data originating from five studies found no significant difference between biofilm communities on different plastic polymers (Oberbeckmann and Labrenz 2020). In another study, in which plastic was deployed in different environmental locations for two weeks, found that the biofilm communities were only different under certain environmental conditions (Oberbeckmann, Kreikemeyer and Labrenz 2018). Accordingly, the initial microbial community was suggested to be the strongest factor for community assembly on plastic (Kesy *et al.* 2019). Likewise, we conclude that the composition of the microbial inoculum drove the community assembly on plastic, but we also found that the type of plastic affected colonization by microbial communities in our setup and each polymer might represent a unique environmental niche to marine microorganisms (Dussud *et al.* 2018b). Even more so, differential biofilm formation on plastic might not only be affected by the chemical composition of the polymer, but also other factors such as additives, the particle size, the surface structure, the degradation state and the resulting hydrophobicity. The biofilm on the plastic is likewise complex. The assembly of the biofilm is driven by the specific characteristics of individual colonizers including cell membrane composition, cell appendages, production of extracellular polymeric substances, the composition of the initial microbiota and maybe most importantly the interaction pathways between the different colonizing microorganisms. Our data from colonization behavior of individual bacterial strains indicated that this phenotype on

different plastic polymers could even be strain-specific. To elucidate how biofilms form on plastic, future work could employ *de novo* assembly of these communities from individual strains as has been successfully employed with other marine particles (Datta *et al.* 2016) and could identify the key drivers of particle colonization. This could contribute to the ultimate aim of understanding the role of marine microorganisms in the fate of plastic debris and possible degradation.

Funding

This work was supported by Innovation Fund of Denmark (case no. 5189-00091B), the VILLUM Foundation and the Danish National Research Foundation (DNRF137).

Acknowledgements

The authors thank Michael Guldager (DTU Mechanical Engineering) for providing plastic pellets, Anna Koza (DTU Biosustain) for support with sequencing and Karen K. Dittmann (DTU Bioengineering) for help and discussion on microbiome analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

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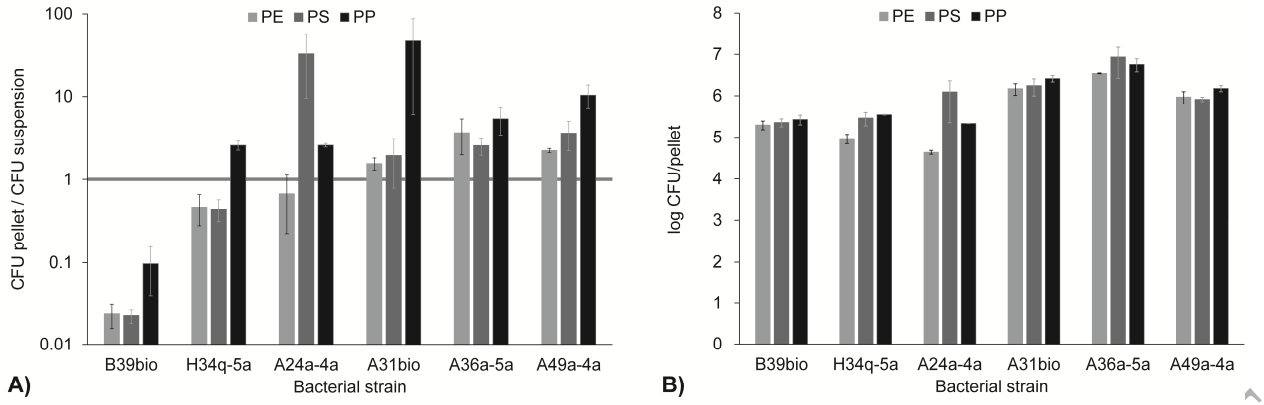

Figure 1. Colonization of six marine bacterial isolates *P. piscicida* B39bio, *P. ulvae* H34q-5a, *P. ulvae* A24a-4a, *Vibrio* sp. A31bio, *Phaeobacter* sp. A36a-5a and *Phaeobacter* sp. A49a-4a on polyethylene, polypropylene and polystyrene pellets after 24 hours (n=2). Cell numbers as A) cells/pellets on pellets and B) ratio of total pellet CFU over total suspension CFU in each sample. The error bars show the standard deviation. See Figure S1 for additional figures.

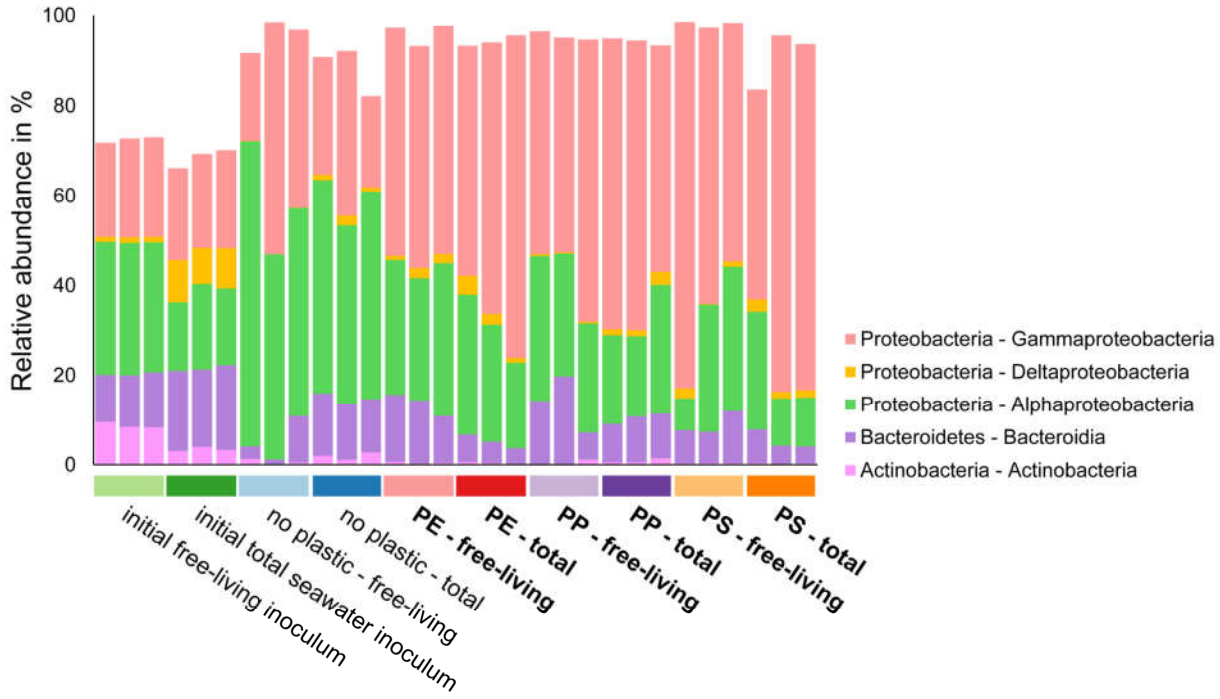

Figure 2. Relative abundance of the most abundant classes in the microbial communities on three plastic types (PE = polyethylene (red), PP = polypropylene (purple), PS = polystyrene (orange)) incubated with either total seawater (dark color) or the free-living fraction (light color), the microbial communities in the free-living (light blue) and total seawater (dark blue) control without plastic and the microbial communities of the initial free-living (light green) and total seawater (dark green) inoculum.

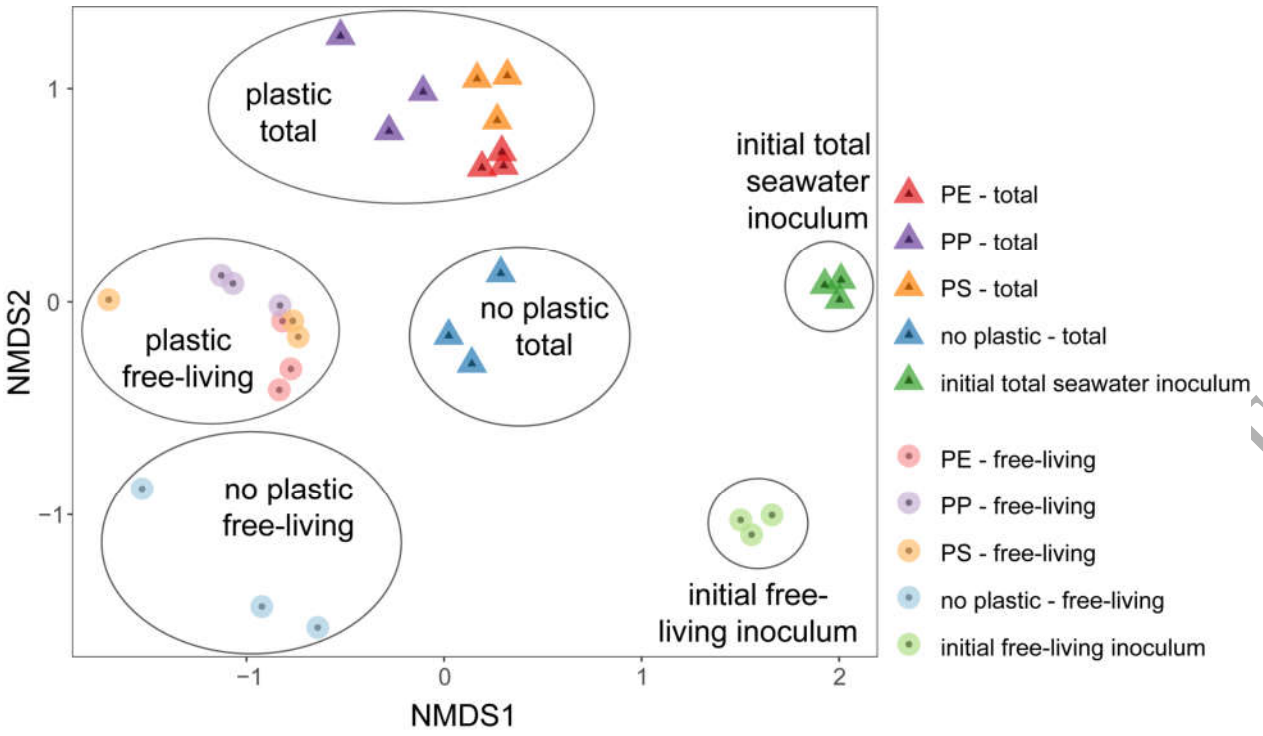

Figure 3. Non-metric multidimensional scaling (NMDS) plot based on the Bray–Curtis distances calculated between individual microbial communities on plastic (PE = polyethylene (green), PP = polypropylene (blue), PS = polystyrene (purple)), in the seawater controls (blue) and the initial inoculum (green) for the total seawater (dark color) and the free-living fraction (light color). Circles around sample types (two different inocula, controls without plastic with two different inocula, samples with plastic with two different inocula) were drawn manually.

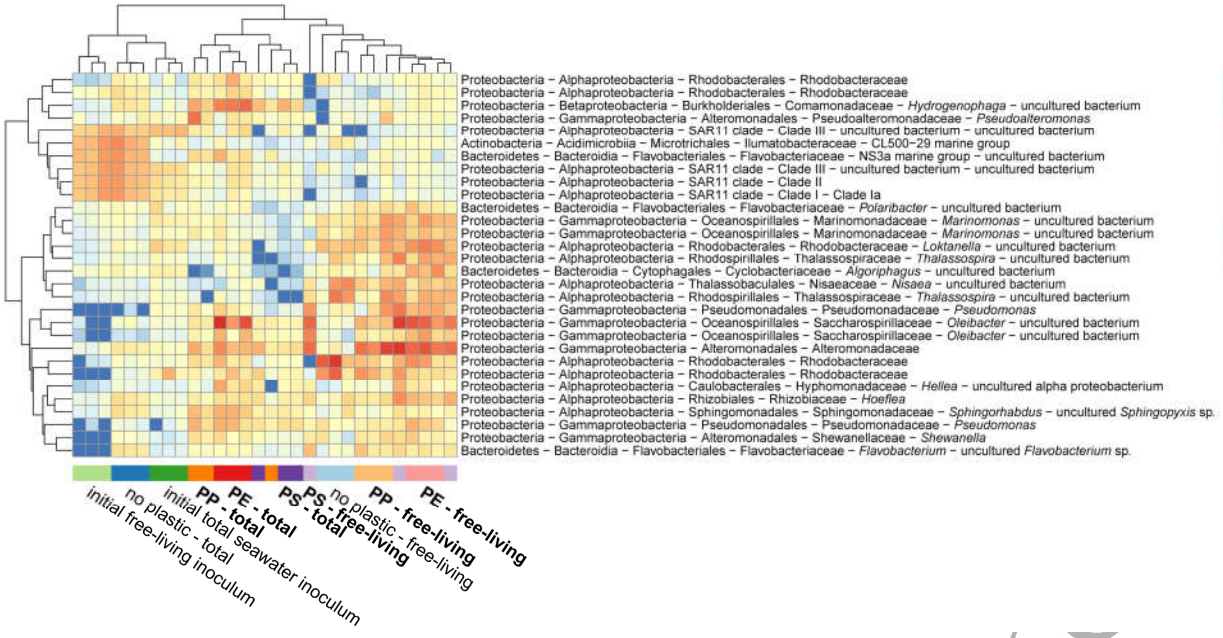

Figure 4. Heatmap indicating the $\log_{10}(x + 1)$-transformed relative abundances of sequences of the 30 most abundant OTUs of the microbial communities on the different plastic types (PE = polyethylene (red), PP = polypropylene (orange), PS = polystyrene (purple)), in the seawater controls (blue) and the initial inoculum (green) for the total seawater (dark color) and the free-living fraction (light color). The SILVA annotations are listed next to the individual OTUs, and the Bray-Curtis distances are represented as dendrograms. Red color in the heatmap indicates the highest abundance and blue the lowest abundance/absence of sequences.