Bacterial Candidates for Colonization and Degradation of Marine Plastic Debris

Roager, Line; Sonnenschein, Eva C.

Published in:
Environmental Science and Technology

Link to article, DOI:
10.1021/acs.est.9b02212

Publication date:
2019

Document Version
Peer reviewed version

Citation (APA):
Bacterial Candidates for Colonization and Degradation of Marine Plastic Debris

Line Roager\textsuperscript{1}, Eva C. Sonnenschein\textsuperscript{1,*}

* corresponding author

\textsuperscript{1} Technical University of Denmark, Department of Biotechnology and Biomedicine, Søltofts Plads 221, 2800 Kgs. Lyngby, Denmark

Marine bacteria; bioremediation; plastic pollution; biodegradation; plastic

ABSTRACT. With the rising plastic pollution in the oceans, research on the plastisphere – the microorganisms interacting with marine plastic debris – has emerged. Microbial communities colonizing plastic have been characterized from several ocean regions and they are distinct from the communities of the surrounding waters and a few plastic-degrading microorganisms have been isolated from other environments. Therefore, we propose that marine microorganisms have adapted to plastic as a surface for colonization and potentially degradation. When comparing the taxonomic patterns of plastic-associated, marine bacteria, recurring groups and families such as the families Erythrobacteraceae and Rhodobacteraceae (Alphaproteobacteria), Flavobacteriaceae
(Bacteriodetes) and the phylum of Cyanobacteria (such as the Phormidium genus) can be identified. Thereby, we provide a perspective on which bacterial candidates could play a role in the colonization and possible degradation of plastic in the oceans due to their occurrence on marine plastic debris. We emphasize the need for extended and reproducible collection of data to assess the existence of a core microbiome or core functionalities of the plastisphere and confirm the capability of these bacterial candidates for biodegradation of plastic. Furthermore, we suggest the next steps in research to elucidate the level of natural bioremediation and the exploitation of bacterial degradative mechanisms of plastic.

1. Introduction

In recent years, the problem of plastic pollution has gained growing public and scientific attention. The global plastic demand increased from 1.5 million tons in the 1950s to 335 million tons in 2016 with an estimated 50% of plastic products being disposable. Due to incorrect disposal or spill during production or transport, plastics can enter the marine environment where they are persistent pollutants. An estimated 4.8 to 12.7 million tons of plastic enter the ocean per year. Today, between 80 and 85% of marine litter is made up of plastic, and both macro- (> 5 mm) and microplastics (< 5 mm) can be a threat to marine wildlife due to entanglement or ingestion. Additionally, plastic additives, climate-relevant trace gases, or dissolved organic carbon leaching from the plastic can – potentially negatively – affect the environment. While abiotic fragmentation of plastic does occur in the ocean due to wave action, oxidation, and photo-oxidation, the actual rate of degradation in the marine environment and the ultimate fate of plastic remains unresolved. The amount of plastic entering the marine system exceeds
the amount of plastic detected in the surface ocean\textsuperscript{16} and therefore, biofouling and biodegradation have been proposed as processes of plastic removal from surface waters.\textsuperscript{13,17} Recently, both fungi and bacteria have been proposed to or even identified to degrade different types of plastic from primarily terrestrial sources.\textsuperscript{17–30} In the marine environment, our current knowledge is primarily limited to the microbial diversity of communities colonizing plastic surfaces\textsuperscript{31–40}. However, marine bacteria are capable of degrading natural hydrocarbons from e.g. oil spills or cellular lipids and are proposed to play a major role in the ocean’s hydrocarbon cycle.\textsuperscript{41,42} Since the ocean is experiencing such high level of plastic pollution, we propose that marine microorganisms may also evolve to utilize this new carbon source. Biodegradation of plastic by microorganisms and their enzymes could present a way of natural bioremediation and possibly affect the global plastic pollution. Additionally, controlled application of these mechanisms could facilitate reduction of the plastic persistence in the environment and could – with the improvements that today’s synthetic biology can provide\textsuperscript{42,44} – be implemented in cell factory design for employment in a circular economy.\textsuperscript{45–48}

With our current state of knowledge, what are the best bacterial candidates for colonization and degradation of marine plastic litter? We will address this question by summarizing the studied bacteria associated with marine plastic debris either as colonizers or as degraders, and give a perspective, on which taxonomic groups are of particular interest for future investigation regarding bacterial biodegradation of the following ‘non-biodegradable’ plastics: polystyrene (PS), polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET). The so-called biodegradable plastic types and associated bacteria will not be further discussed in the present work (recently reviewed by Emadian et al.\textsuperscript{49}), as our target is commercial, synthetic
plastic that constitutes the vast majority of plastic debris in the oceans.\textsuperscript{50} Since fungi are largely outnumbered by bacteria in the oceans,\textsuperscript{51,52} this work focuses on bacteria.

2. Marine Bacteria colonizing Plastic Debris

Colonization of marine plastic debris by microorganisms was first described in 1972\textsuperscript{53,54} and in recent years, an increasing number of studies have investigated the composition of microbial communities with regards to their taxonomic units. Bacterial communities inhabiting the surfaces of marine plastic debris differ significantly from the communities in the pelagic waters around them or on other particle types.\textsuperscript{31,33,37–40} Certain bacterial groups belonging to the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria (Table 1) appear to be enriched on plastic more often than others, suggesting that this is an ecological niche beneficial for specific taxonomic groups and indicating a potential metabolic adaptation (e.g. attachment, chemotaxis, additive resistance, degradation) to the material. If these bacteria have developed enzymatic mechanisms for the degradation of plastic, these bacterial groups are of specific interest for bioremediation and bioengineering purposes.

Table 1. Bacterial families re-occurring on marine plastic debris as identified by sequenced-based analysis of microbial diversity, plastic type, sampling site.

<table>
<thead>
<tr>
<th>family</th>
<th>order</th>
<th>class</th>
<th>plastic type</th>
<th>sampling site</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavobacteriaceae</td>
<td>Flavobacteriales</td>
<td>Flavo-bacteria\textsuperscript{1}</td>
<td>PE, PET, PP, PS</td>
<td>North Sea, Baltic Sea, Mediterranean Sea, North Atlantic Ocean, Humber Estuary, North Pacific Ocean</td>
<td>33,35–37,39,55–57</td>
</tr>
<tr>
<td>Order</td>
<td>Class</td>
<td>Subclass</td>
<td>PE, PET, ND</td>
<td>Locations</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>------------------------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Saprospiraceae</td>
<td>Sphingobacteriales</td>
<td>Sphingobacteria</td>
<td>PE, PET, ND</td>
<td>Yangtze Estuary, North Atlantic Ocean, North Pacific Ocean, Baltic Sea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyphomonadaceae</td>
<td>Rhodobacterales</td>
<td>Alpha-proteobacteria</td>
<td>PE, PP, PS</td>
<td>North Sea, North Atlantic Ocean, Baltic Sea, Mediterranean Sea, North Pacific Ocean</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodobacteraceae</td>
<td>Rhodobacterales</td>
<td>Alpha-proteobacteria</td>
<td>PE, PET, PP, PS, ND</td>
<td>Baltic Sea, Mediterranean Sea, North Atlantic Ocean, North Pacific Ocean, North Sea, Yangtze Estuary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrobacteraceae</td>
<td>Sphingomonadales</td>
<td>Alpha-proteobacteria</td>
<td>PE, PP, ND</td>
<td>North Sea, Mediterranean Sea, North Atlantic Ocean, Yangtze Estuary, Baltic Sea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonadaceae</td>
<td>Sphingomonadales</td>
<td>Alpha-proteobacteria</td>
<td>PE, PP, PS, ND</td>
<td>North Atlantic Ocean, Yangtze Estuary, Baltic Sea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comamonadaceae</td>
<td>Burkholderiales</td>
<td>Beta-proteobacteria</td>
<td>PET, PS, ND</td>
<td>Baltic Sea, North Atlantic Ocean, North Pacific Ocean, Yangtze Estuary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcanivoracaceae</td>
<td>Oceanospirillales</td>
<td>Gamma-proteobacteria</td>
<td>PE, PET, PS</td>
<td>North Atlantic Ocean, Mediterranean Sea, North Sea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoalteromonadaceae</td>
<td>Alteromonadales</td>
<td>Gamma-proteobacteria</td>
<td>PE, PP, ND, ND</td>
<td>North Sea, North Atlantic Ocean, Ocean, Yangtze Estuary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceanospirillaceae</td>
<td>Oceanospirillales</td>
<td>Gamma-proteobacteria</td>
<td>PE, PET, PS</td>
<td>North Atlantic Ocean, Mediterranean Sea, North Sea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.1 Proteobacteria. The globally most abundant phylum of Proteobacteria also comprises the most observed phylum on marine plastic debris compared to seawater.\textsuperscript{36,38,40,56,57} This phylum has observed with a higher abundance on plastic surfaces compared to glass and organic surfaces.\textsuperscript{40} Within this phylum, the classes of Alphaproteobacteria, Gammaproteobacteria and in some cases, of Betaproteobacteria and Deltaproteobacteria are enriched on plastic samples.\textsuperscript{39,56,59,61} The alphaproteobacterial family of Hyphomonadaceae contains members known as methylotrophs, hydrocarbon degraders, and members isolated from hydrocarbon-enriched environments and are also abundant colonizers of PP, PS, and PE.\textsuperscript{31,33,37,39,40,59} Some species have even developed a “holdfast” dedicated and essential for surface attachment demonstrating their adaptation to a sessile life stage.\textsuperscript{62} Compared to colonization of wooden pellets, the Hyphomonadaceae family was significantly associated with PE and PS.\textsuperscript{37} Another alphaproteobacterial family commonly colonizing marine plastic is Rhodobacteraceae.\textsuperscript{31,33,35,37,39,40,55,57–60} This family is known as “initial colonizers” of various substrates in the marine environment\textsuperscript{63} indicating a non-specificity for plastic. In a recent study, this family was found more frequently on marine plastic debris compared to organic particles.\textsuperscript{33} The common marine plastic-colonizing family of Erythrobacteraceae has been identified on PP, PS, PE and PET and was found on both macro- and microplastics.\textsuperscript{31,33,36,37,40,58,59} They are known to be capable of degrading polycyclic aromatic hydrocarbons (PAHs) that can be associated with plastic and could explain their increased abundance\textsuperscript{64}. Erythrobacteraceae have been identified as
significantly enriched on PE compared to wooden pellets, but has also been found in high abundances on organic particles isolated from the marine environment compared to non-specific plastic types.33

Within the Gammaproteobacteria, orders like the Pseudomonadales have been highlighted by several research groups.31,35,37 Species of the *Pseudomonas* genus isolated from soil and waste sites have previously been identified as degraders of plastic,18,19,25,28,65,66 making this genus of specific interest for future research. The order of Oceanospirillales has also been found in association with plastic by several studies,35,55–57,67 and the family of Alcanivoraceae stands out as significantly associated with PE, PET and PS.35,56 Members of this family were more frequently found on plastics compared to glass beads.35 The type genus of this family, *Alcanivorax*, forms biofilms in marine environments and has genomic capacity to degrade several oil-derived hydrocarbons.68 Members of the genus *Vibrio* were also detected on plastic surface samples when compared to the free-living community, however they are also present on many other marine surfaces.31,34,38,55,69 Strains of this group have been found to degrade PAHs70 and the group is very well studied for its ability to degrade the natural polymer chitin.71,72

The most prevalent order colonizing marine plastic debris within the Betaproteobacteria is the Burkholderiales.37,40,58,59 This order has been found to be significantly enriched on plastic beads compared to glass beads and cellulose.40 An especially abundant genus is *Hydrogenophaga*, of which species have been observed as colonizers of PE, PP, and PS.37,40 Species belonging to the Burkholderiales order have previously been shown to degrade petroleate derivatives.73 The *Ideonella sakaiensis* strain capable of degrading PET26 belongs to the Betaproteobacteria class, but no strains of this species have been detected as colonizers of marine plastic debris.
One order within the class of Deltaproteobacteria has been reported several times as colonizers of plastic in the oceans, the Myxococcales order.\textsuperscript{31,35,37,40} This order is known for its ability to produce hydrolytic enzymes and decompose various polymers,\textsuperscript{74} which is of interest regarding their potential degradation of plastic polymers.

2.2 Bacteriodetes. The Bacteriodetes phylum can be enriched on plastic surfaces.\textsuperscript{31,36,38,59} Especially, the order of Flavobacteriales has been found as colonizers on PS, (LD)PE, PP, and PET.\textsuperscript{31,33,35–37,39,55–57} The genus \textit{Flavobacterium} has previously been associated with plastic in other environments than the marine.\textsuperscript{56,65} Another genus, \textit{Tenacibaculum}, has repeatedly been found in significantly high abundances on plastic surfaces in marine environments.\textsuperscript{31,35–37,39,55,59} This genus contains species known as degraders of the polyester polycaprolactone, which is a so-called biodegradable plastic type.\textsuperscript{75} A species of Flavobacteriales, \textit{Ulvibacter litoralis}, was found exclusively on plastic particles compared to bacteria isolated from the water column and organic particles.\textsuperscript{33} Additionally, the Flavobacteriaceae family was found more frequently on PET particles compared to glass beads\textsuperscript{35} as well as on PE and PS compared to wooden pellets.\textsuperscript{37} The order Sphingobacteriales has also frequently been identified as colonizers of plastic, with the \textit{Lewinella} genus as the most prevalent one, but also many bacteria unclassified at genus level belonging to the order have been associated with different types of plastics such as PS, PE, PET, and PP.\textsuperscript{31,35,39,40,58,59} Some species belonging to the Sphingobacteriales order are capable of metabolizing PAHs,\textsuperscript{76} making them particularly interesting with regards to potential bioremediation of specific plastic components.

2.3 Firmicutes and Cyanobacteria. The Bacillaceae family of the Firmicutes phylum was identified as significantly enriched on marine PS samples by Syranidou \textit{et al.}\textsuperscript{56} in accordance with the marine bacterial degraders of plastic already found (\textbf{Table 2}). Cyanobacteria have been
observed as significantly enriched on various types of plastic, e.g. *Phormidium* species. They have been found on PE, PET, and PP samples and are known to degrade hydrocarbons. Since Cyanobacteria are photosynthetic organisms, the benefit of higher sunlight exposure on floating plastic pieces might however be the actual reason for their enrichment on plastic debris.

### 3. Marine Plastic-Degrading Bacteria

In 2016, Yoshida *et al.* isolated a novel bacterial strain, *Ideonella sakaiensis* 201-F6, from a PET bottle recycling site that is able to both degrade and assimilate highly crystallized PET. In contrast to this extraordinary finding and subsequent optimization of the PETase enzyme, our knowledge on plastic-degrading marine bacteria and their enzymes is very limited and awaits confirmation of the metabolic mechanisms. The limited information available was recently reviewed by Jacquin *et al.* Whereas biodegradation of PET under aerobic conditions is known to be initiated by the PETase enzyme, the most common biodegradation pathway for PS is initiated by a styrene monooxygenase. For PP and PE such information is not available, but it is generally thought that PE biodegradation is initiated by either biotic or abiotic oxidation of the PE chain, and oxidoreductases, laccases and an alkane hydroxylase have been found to play central roles. Sudhakar *et al.* discovered two marine *Bacillus* strains capable of degrading low-density and high-density polyethylene (LDPE and HDPE) in 2008 (Table 2). However, this degradation happened at very low rates with a maximum weight loss of 10% and 3.5% for LDPE and HDPE, respectively, after 1 year. If the plastic samples were pretreated thermally, the percentage of weight loss increased significantly, indicating that preceding weathering of plastic might make it more accessible for biodegradation by bacteria. Further marine plastic degraders belonging to the *Bacillus* genus have been isolated from mangrove sediment. A *B. cereus* strain
was able to degrade both PE, PET and PS microplastics at rates of 1.6%, 6.6%, and 7.4% weight loss, respectively, in 40 days. A *B. gottheilii* strain degraded PE, PET, PS, and PP at rates of 6.2%, 3.0%, 5.8%, and 3.6% weight loss, respectively, in 40 days. A third strain, *Bacillus sp.* 27, was able to degrade PP at a rate of 4% in 40 days. Two other marine Bacilli, *B. pumilus* M27 and *B. subtilis* H1584, isolated from coastal waters of the Arabian Sea were able to degrade LDPE at rates of 1.5% and 1.75% in 30 days, respectively. Gram-positive plastic-degrading bacterial isolates include the marine *Rhodococcus sp.* strain 36 (degradation of PP with 6.4% weight loss in 40 days) and the *Kocuria palustris* strain M16 (degradation of LDPE with 1% weight loss in 30 days).

**Table 2. List of suggested plastic-degrading marine bacteria, degraded plastic type, degradation rate and origin of isolate.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Species and strain</th>
<th>Degraded plastic type</th>
<th>% Weight loss</th>
<th>Duration of experiment</th>
<th>% Weight loss per year</th>
<th>Origin of bacterial sample</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td><em>Kocuria palustris</em></td>
<td>LDPE</td>
<td>1</td>
<td>30 days</td>
<td>12.2</td>
<td>Seawater, Arabian Sea</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>M16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rhodococcus sp.</em></td>
<td>PP</td>
<td>6.4</td>
<td>40 days</td>
<td>58.4</td>
<td>Mangrove sediment, Malaysia</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacilli</td>
<td><em>Bacillus cereus</em></td>
<td>LDPE, HDPE</td>
<td>5, 2</td>
<td>1 year</td>
<td>5.0, 2.0</td>
<td>Indian Ocean</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>BF20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Additionally, microbial consortia with plastic-degrading activities have been investigated in the laboratory. A synthetic community of four *Vibrio* strains showed biodegradation of polyvinyl alcohol-low linear density polyethylene (PVA-LLDPE). Another consortium developed by acclimatizing and bioaugmenting an indigenous community isolated from seawater was able to degrade PE film with 19% weight loss in 6 months. The effect of indigenous microbial consortia on plastic submerged in seawater was detected as percentage weight loss of up to 2.5%
in LDPE in six months,\textsuperscript{85} 1.6\% in HDPE in 12 months,\textsuperscript{86} 0.6\% in PP in six months,\textsuperscript{85} 30\% in nylon 4 in 3 weeks,\textsuperscript{87} 0.69\% in polycarbonate (PC) in 12 months.\textsuperscript{86} While microorganisms colonizing the plastic surfaces were observed,\textsuperscript{85,86} it should be noted that the respective percentage weight losses cannot be exclusively attributed to biodegradation by these microorganisms. Nevertheless, the findings indicate that the microbial communities colonizing marine plastic litter are of interest in the search for plastic-degrading marine bacteria; however, the identification and characterization of plastic-degrading pathways from the marine environment remains to be awaited.

4. Discussion

Certain bacterial orders and families are repeatedly highlighted in an increasing number of studies on the microbial communities colonizing marine plastic debris, giving rise to the term “plastisphere” for this distinct habitat.\textsuperscript{31} This term has, however, also been challenged due to variability in the plastisphere in regard to geographical and environmental differences.\textsuperscript{35–37,58} Harrison \textit{et al.}\textsuperscript{88} determined the taxonomy of bacteria colonizing LDPE in marine sediment in an estuary and found two main genera; \textit{Colwellia} and \textit{Arcobacter}, which have not been found by studies sampling plastic debris in the pelagic water column. It has been proposed that the core microbiome differs depending on type or age of the plastic substrate, broadening the term “plastisphere” for a specific microbial composition on marine plastic.\textsuperscript{38,59} On the other hand, researchers have demonstrated the significant difference between the microbial communities on marine plastic litter and the surrounding seawater and sediment at different geographical locations such as the North Pacific Subtropical Gyre, the North Atlantic Subtropical Gyre, and the North Sea.\textsuperscript{31,35,38,39} Furthermore, it was shown that bacterial communities attached to plastic particles differed from those attached to a range of marine organic particles\textsuperscript{33} as well as cellulose
and glass beads. However, others have found no significant difference in community composition of bacteria colonizing plastic particles and glass beads, indicating that the communities might form on any hard surface, not exclusively on plastic surfaces. This is a very important aspect to be addressed in future research within this area. The plastic-associated communities have certain bacterial families in common even across geographical locations (Table 1), supporting the idea of a core microbiome native to the “plastisphere”. Furthermore, certain genes or taxon-associated functionalities are commonly enriched in the “plastisphere”. Often, this includes metabolic pathways associated with degradation of xenobiotics, indicating that bacteria colonizing plastic surfaces have common genomic traits and could metabolically respond to the plastic, its additives or other adsorbed pollutants. Additionally, some of the most commonly identified bacterial families associated with plastics, such as Rhodobacteraceae and Hyphomonadaceae, also contain hydrocarbon-degrading members or members previously associated with oil spills. As stated previously, the research community should however be aware of environmental, geographical and surface-dependent differences in the microbial community in future research efforts.

5. Perspectives and Outlook for Marine Plastic Biodegradation

While this perspective attempts to give an overview of a potential core microbiome on marine plastic surfaces as well as marine plastic-degrading bacteria based on the presently available data, it is evident that more data and research are needed. The current knowledge provides indications for a core microbiome in the plastisphere, but in order to understand potential degradation processes and to trace and potentially exploit marine plastic-degrading bacteria, several steps of research are needed:
5.1 Collection of Data. Currently available data originated from very limited geographic areas in the Northern hemisphere (Figure 1). More data from different geographical locations, particularly the continuously exposed plastic-polluted ocean areas such as the major ocean gyres, as well as seasons and environments (sediment vs. pelagic waters) characterizing the unique bacterial communities on plastics are needed. The next-generation sequencing approach of the hypervariable regions of the 16S rRNA gene, first introduced on the plastisphere by Zettler et al.,\textsuperscript{31} will be sufficient to confirm the core microbiome on plastic. Efforts should be adapted to protocols of the Earth Microbiome Project (http://www.earthmicrobiome.org/) to produce consistent data comparable across different experiments and sampling sites.\textsuperscript{89} Furthermore, future studies should collect data on colonization of non-plastic surfaces along with plastic surfaces for comparison of the bacterial communities, similar to the approach of Oberbeckmann et al.\textsuperscript{35} Time series of plastic colonization patterns should be analyzed similar to those conducted on other marine particles such as chitin to elucidate the different roles of primary, secondary etc. colonizers on the material and the overall biofilm structure.\textsuperscript{90} These studies could clarify if the bacteria in the plastisphere actually degrade or modify the material or simply use it as a surface and grow on more readily available photosynthetically-produced carbohydrates. Metagenomic or metatranscriptomic data that not only provide information on taxonomic, but also functional units (present or expressed genes) as described by Bryant et al.\textsuperscript{39} will be important to assess the potential of bacterial colonizers to degrade the plastic and discover novel plastic-degrading pathways.\textsuperscript{91} This data can furthermore be utilized to screen for novel homologs of known plastic-degrading enzymes.\textsuperscript{92,93}
Figure 1. World map with sampling sites of currently available biodiversity data on marine plastic debris (blue rings).\textsuperscript{31,33,35–40,55–59,88}

5.2 Assessment of a Core Plastic-Degrading Microbiome. Based on sequence data collected from marine plastic samples, a core microbiome for or core functionalities on marine plastics should be investigated. This core microbiome might cover general marine plastic litter or divide into sub-groups by season, geography or plastic type based on the statistical significance levels of data collected.

5.3 Search for Plastic-Degrading Bacteria or Consortia. With a core microbiome assessed, a qualified search for marine plastic-degrading bacteria or consortia can be conducted. This research will most likely be based on culture-dependent methods or on bioinformatic analyses of -omic data from marine plastic samples. Ultimately, any bioinformatic results will be followed up by culture-dependent methods by necessity to establish whether plastic degradation occurs \textit{in vivo}. The core microbiome and associated degradation tests will furthermore indicate if bacteria
indeed contribute to the fate of plastic in the marine environment and if natural bioremediation does occur.

### 5.4 Optimization and Implementation of Plastic-Degrading Bacteria and their Enzymes.

Following the isolation of plastic-degrading bacteria, the enzymes involved in plastic degradation can be characterized, investigated, and optimized as previously shown.\textsuperscript{26,46}

Investigation of the evolutionary origin of plastic-degrading enzymes will contribute to the search for other plastic-degrading enzymes and development strategies.\textsuperscript{26,46} Optimization of enzymes through acclimatization experiments, adaptive laboratory evolution and genetic or protein engineering can also be performed to increase efficiency.\textsuperscript{46,93,94} Utilization of plastic-degrading enzymes for bioremediation purposes must go through careful risk assessment and utilization of bacterial strains in the environment should be closely monitored and risk assessed to control spread of their bioactivities. Biofilters entrapping live, plastic-degrading bacteria could however provide an applicable method of bioremediation.\textsuperscript{95} Application of plastic-degrading pathways for the utilization of plastic as a feedstock for cell factories and their most appropriate integration into host metabolism can be guided through metabolic reconstructions of the production hosts\textsuperscript{96} and scaled up in bioreactors.\textsuperscript{97}

### 5.5 Interdisciplinary Work.

A key component of future work on biodegradation of plastic will also be the collaboration of scientists from various disciplines next to microbiologists including, but not limited to: Bioinformaticians and structural biologists to develop \textit{in silico} methods for the reliable identification of putative plastic-degrading enzymes and subsequent precursor/monomer processing; material scientists and chemists to design fluorescently or isotope labelled polymers or polymer stains\textsuperscript{98} to trace biodegradation processes; analytical chemists, ecotoxicologists and oceanographers to provide the analytical and computational
techniques to accurately measure and model concentration and distribution of plastic particles in
the environment; experts in methods to visually describe the biofilms on the plastic surface and
the decay of the polymer itself such as electron, confocal, and atomic force microscopy, Fourier
Transform Infrared (FTIR) and Raman spectroscopy.

Today, we have only scratched the surface of understanding the fate and impact of plastic in
the oceans, however, it is already apparent that plastic does affect the environment including the
bacteria associated with its surface. It will require joint scientific, interdisciplinary efforts to
assess if these bacteria influence the transport of plastic, change its polymeric structure or even
degradate it to use it as carbon source, but if this is the case, these bacteria would provide an
innovative, promising resource for future bioremediation and biotechnology.

AUTHOR INFORMATION

Corresponding Author

* Eva C. Sonnenschein; e-mail: evaso@bio.dtu.dk; Technical University of Denmark,
Department of Biotechnology and Biomedicine, Søltofts Plads 221, DK-2800 Kgs. Lyngby,
Denmark

ORCID

Line Roager 0000-0002-7033-7309
Eva C. Sonnenschein 0000-0001-6959-5100

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval
to the final version of the manuscript.
ACKNOWLEDGMENT

The authors thank Sonja Oberbeckmann and Nikolaus Sonnenschein for critical comments on the manuscript.

ABBREVIATIONS

LDPE low density polyethylene, HDPE high density polyethylene, PAH polycyclic aromatic hydrocarbon, PC polycarbonate, PE polyethylene, PET polyethylene terephthalate, PP polypropylene, PS polystyrene, PVA-LLDPE polyvinyl alcohol-low linear density polyethylene

REFERENCES


Cozar, A.; Echevarria, F.; Gonzalez-Gordillo, J. I.; Irigoien, X.; Ubeda, B.; Hernandez-


(20) Nakajima-Kambe, T.; Onuma, F.; Kimpara, N.; Nakahara, T. Isolation and Characterization


Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; et al. Deep-Sea Oil Plume Enriches


Pollet, T.; Berdjeb, L.; Garnier, C.; Durrieu, G.; Poupon, C. Le; Misson, B.; Briand, J.-F.


Jacquin, J.; Cheng, J.; Odobel, C.; Pandin, C.; Conan, P.; Pujo-Pay, M.; Barbe, V.;


(98) Erni-Cassola, G.; Gibson, M. I.; Thompson, R. C.; Christie-Oleza, J. A. Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 Mm