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1 Bacterial Candidates for Colonization and 2 Degradation of Marine Plastic Debris

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7

8 Marine bacteria; bioremediation; plastic pollution; biodegradation; plastic

9

10 **ABSTRACT.** With the rising plastic pollution in the oceans, research on the plastisphere – the
11 microorganisms interacting with marine plastic debris – has emerged. Microbial communities
12 colonizing plastic have been characterized from several ocean regions and they are distinct from
13 the communities of the surrounding waters and a few plastic-degrading microorganisms have been
14 isolated from other environments. Therefore, we propose that marine microorganisms have
15 adapted to plastic as a surface for colonization and potentially degradation. When comparing the
16 taxonomic patterns of plastic-associated, marine bacteria, recurring groups and families such as
17 the families Erythrobacteraceae and Rhodobacteraceae (Alphaproteobacteria), Flavobacteriaceae

18 (Bacterioidetes) and the phylum of Cyanobacteria (such as the *Phormidium* genus) can be
19 identified. Thereby, we provide a perspective on which bacterial candidates could play a role in
20 the colonization and possible degradation of plastic in the oceans due to their occurrence on marine
21 plastic debris. We emphasize the need for extended and reproducible collection of data to assess
22 the existence of a core microbiome or core functionalities of the plastisphere and confirm the
23 capability of these bacterial candidates for biodegradation of plastic. Furthermore, we suggest the
24 next steps in research to elucidate the level of natural bioremediation and the exploitation of
25 bacterial degradative mechanisms of plastic.

26

27 **1. Introduction**

28 In recent years, the problem of plastic pollution has gained growing public and scientific
29 attention. The global plastic demand increased from 1.5 million tons in the 1950s to 335 million
30 tons in 2016¹ with an estimated 50% of plastic products being disposable². Due to incorrect
31 disposal or spill during production or transport, plastics can enter the marine environment where
32 they are persistent pollutants.^{3,4} An estimated 4.8 to 12.7 million tons of plastic enter the ocean
33 per year.⁵ Today, between 80 and 85% of marine litter is made up of plastic,^{6,7} and both macro-
34 (> 5 mm) and microplastics (< 5 mm) can be a threat to marine wildlife due to entanglement or
35 ingestion.^{2,8} Additionally, plastic additives, climate-relevant trace gases, or dissolved organic
36 carbon leaching from the plastic can – potentially negatively – affect the environment.^{8–11} While
37 abiotic fragmentation of plastic does occur in the ocean due to wave action, oxidation, and
38 photo-oxidation,¹² the actual rate of degradation in the marine environment and the ultimate fate
39 of plastic remains unresolved.^{2,13–15} The amount of plastic entering the marine system exceeds

40 the amount of plastic detected in the surface ocean¹⁶ and therefore, biofouling and
41 biodegradation have been proposed as processes of plastic removal from surface waters.^{13,17}
42 Recently, both fungi and bacteria have been proposed to or even identified to degrade different
43 types of plastic from primarily terrestrial sources.¹⁷⁻³⁰ In the marine environment, our current
44 knowledge is primarily limited to the microbial diversity of communities colonizing plastic
45 surfaces³¹⁻⁴⁰. However, marine bacteria are capable of degrading natural hydrocarbons from e.g.
46 oil spills or cellular lipids and are proposed to play a major role in the ocean's hydrocarbon
47 cycle.^{41,42} Since the ocean is experiencing such high level of plastic pollution, we propose that
48 marine microorganisms may also evolve to utilize this new carbon source. Biodegradation of
49 plastic by microorganisms and their enzymes could present a way of natural bioremediation and
50 possibly affect the global plastic pollution. Additionally, controlled application of these
51 mechanisms could facilitate reduction of the plastic persistence in the environment and could –
52 with the improvements that today's synthetic biology can provide^{43,44} – be implemented in cell
53 factory design for employment in a circular economy.⁴⁵⁻⁴⁸

54 With our current state of knowledge, what are the best bacterial candidates for colonization
55 and degradation of marine plastic litter? We will address this question by summarizing the
56 studied bacteria associated with marine plastic debris either as colonizers or as degraders, and
57 give a perspective, on which taxonomic groups are of particular interest for future investigation
58 regarding bacterial biodegradation of the following 'non-biodegradable' plastics: polystyrene
59 (PS), polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET). The so-
60 called biodegradable plastic types and associated bacteria will not be further discussed in the
61 present work (recently reviewed by Emadian et al.⁴⁹), as our target is commercial, synthetic

62 plastic that constitutes the vast majority of plastic debris in the oceans.⁵⁰ Since fungi are largely
 63 outnumbered by bacteria in the oceans,^{51,52} this work focuses on bacteria.

64 **2. Marine Bacteria colonizing Plastic Debris**

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

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

family	order	class	plastic type	sampling site	reference
Flavobacteriaceae	Flavobacteriales	Flavo-bacteria ¹	PE, PET, PP, PS	North Sea, Baltic Sea, Mediterranean Sea, North Atlantic Ocean, Humber Estuary, North Pacific Ocean	33,35-37,39,55-57

Saprospiraceae	Sphingobacteriales	Sphingobacteria ¹	PE, PET, ND	Yangtze Estuary, North Atlantic Ocean, North Pacific Ocean, Baltic Sea	31,35,39,40,58
Hyphomonadaceae	Rhodobacterales	Alpha-proteobacteria ²	PE, PP, PS	North Sea, North Atlantic Ocean, Baltic Sea, Mediterranean Sea, North Pacific Ocean	31,33,37,39,40,59
Rhodobacteraceae	Rhodobacterales	Alpha-proteobacteria ²	PE, PET, PP, PS, ND	Baltic Sea, Mediterranean Sea, North Atlantic Ocean, North Pacific Ocean, North Sea, Yangtze Estuary	31,33,35,37,39,55,57-60
Erythrobacteraceae	Sphingomonadales	Alpha-proteobacteria ²	PE, PP, ND	North Sea, Mediterranean Sea, North Atlantic Ocean, Yangtze Estuary, Baltic Sea	31,33,36,37,40,58,59
Sphingomonadaceae	Sphingomonadales	Alpha-proteobacteria ²	PE, PP, PS, ND	North Atlantic Ocean, Yangtze Estuary, Baltic Sea	37,40,58,59
Comamonadaceae	Burkholderiales	Beta-proteobacteria ²	PET, PS, ND	Baltic Sea, North Atlantic Ocean, Yangtze Estuary	37,40,58,59
Alcanivoracaceae	Oceanospirillales	Gamma-proteobacteria ²	PE, PET, PS	North Sea, Mediterranean Sea	35,56
Pseudoalteromonadaceae	Alteromonadales	Gamma-proteobacteria ²	PE, PP, PS, ND	North Sea, North Atlantic Ocean, Yangtze Estuary	31,38,55,58
Oceanospirillaceae	Oceanospirillales	Gamma-proteobacteria ²	PE, PET, PS	North Atlantic Ocean, Mediterranean Sea, North Sea	31,35,56,57

Vibrionaceae	Vibrionales	Gamma- proteobacteria ²	PE, PP, PS	North Sea, North Atlantic ^{31,38,55} Ocean
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78 ¹ phylum of Bacteroidetes; ² phylum of Proteobacteria; ND = not defined.

79

80 **2.1 Proteobacteria.** The globally most abundant phylum of Proteobacteria also comprises the
81 most observed phylum on marine plastic debris compared to seawater.^{36,38,40,56,57} This phylum
82 has observed with a higher abundance on plastic surfaces compared to glass and organic
83 surfaces.⁴⁰ Within this phylum, the classes of Alphaproteobacteria, Gammaproteobacteria and in
84 some cases, of Betaproteobacteria and Deltaproteobacteria are enriched on plastic
85 samples.^{39,56,59,61} The alphaproteobacterial family of Hyphomonadaceae contains members
86 known as methylotrophs, hydrocarbon degraders, and members isolated from hydrocarbon-
87 enriched environments and are also abundant colonizers of PP, PS, and PE.^{31,33,37,39,40,59} Some
88 species have even developed a “holdfast” dedicated and essential for surface attachment
89 demonstrating their adaptation to a sessile life stage.⁶² Compared to colonization of wooden
90 pellets, the Hyphomonadaceae family was significantly associated with PE and PS.³⁷ Another
91 alphaproteobacterial family commonly colonizing marine plastic is
92 Rhodobacteraceae.^{31,33,35,37,39,40,55,57–60} This family is known as “initial colonizers” of various
93 substrates in the marine environment⁶³ indicating a non-specificity for plastic. In a recent study,
94 this family was found more frequently on marine plastic debris compared to organic particles.³³
95 The common marine plastic-colonizing family of Erythrobacteraceae has been identified on PP,
96 PS, PE and PET and was found on both macro- and microplastics.^{31,33,36,37,40,58,59} They are known
97 to be capable of degrading polycyclic aromatic hydrocarbons (PAHs) that can be associated with
98 plastic and could explain their increased abundance⁶⁴. Erythrobacteraceae have been identified as

99 significantly enriched on PE compared to wooden pellets,³⁷ but has also been found in high
100 abundances on organic particles isolated from the marine environment compared to non-specific
101 plastic types.³³

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

114 The most prevalent order colonizing marine plastic debris within the Betaproteobacteria is the
115 Burkholderiales.^{37,40,58,59} This order has been found to be significantly enriched on plastic beads
116 compared to glass beads and cellulose.⁴⁰ An especially abundant genus is *Hydrogenophaga*, of
117 which species have been observed as colonizers of PE, PP, and PS.^{37,40} Species belonging to the
118 Burkholderiales order have previously been shown to degrade petroleate derivatives.⁷³ The
119 *Ideonella sakaiensis* strain capable of degrading PET²⁶ belongs to the Betaproteobacteria class,
120 but no strains of this species have been detected as colonizers of marine plastic debris.

121 One order within the class of Deltaproteobacteria has been reported several times as
122 colonizers of plastic in the oceans, the Myxococcales order.^{31,35,37,40} This order is known for its
123 ability to produce hydrolytic enzymes and decompose various polymers,⁷⁴ which is of interest
124 regarding their potential degradation of plastic polymers.

125 **2.2 Bacteroidetes.** The Bacteroidetes phylum can be enriched on plastic surfaces.^{31,36,38,59}
126 Especially, the order of Flavobacteriales has been found as colonizers on PS, (LD)PE, PP, and
127 PET.^{31,33,35-37,39,55-57} The genus *Flavobacterium* has previously been associated with plastic in
128 other environments than the marine.^{56,65} Another genus, *Tenacibaculum*, has repeatedly been
129 found in significantly high abundances on plastic surfaces in marine environments.^{31,35-37,39,55,59}
130 This genus contains species known as degraders of the polyester polycaprolactone, which is a so-
131 called biodegradable plastic type.⁷⁵ A species of Flavobacteriales, *Ulvibacter litoralis*, was found
132 exclusively on plastic particles compared to bacteria isolated from the water column and organic
133 particles.³³ Additionally, the Flavobacteriaceae family was found more frequently on PET
134 particles compared to glass beads³⁵ as well as on PE and PS compared to wooden pellets.³⁷ The
135 order Sphingobacteriales has also frequently been identified as colonizers of plastic, with the
136 *Lewinella* genus as the most prevalent one, but also many bacteria unclassified at genus level
137 belonging to the order have been associated with different types of plastics such as PS, PE, PET,
138 and PP.^{31,35,39,40,58,59} Some species belonging to the Sphingobacteriales order are capable of
139 metabolizing PAHs,⁷⁶ making them particularly interesting with regards to potential
140 bioremediation of specific plastic components.

141 **2.3 Firmicutes and Cyanobacteria.** The Bacillaceae family of the Firmicutes phylum was
142 identified as significantly enriched on marine PS samples by Syranidou *et al.*⁵⁶ in accordance
143 with the marine bacterial degraders of plastic already found (**Table 2**). Cyanobacteria have been

144 observed as significantly enriched on various types of plastic,^{33,36,39,59} e.g. *Phormidium* species
145 have been found on PE, PET, and PP samples^{31,33,35,36,39,58,59} and are known to degrade
146 hydrocarbons.⁷⁷ Since Cyanobacteria are photosynthetic organisms, the benefit of higher sunlight
147 exposure on floating plastic pieces might however be the actual reason for their enrichment on
148 plastic debris.

149 **3. Marine Plastic-Degrading Bacteria**

150 In 2016, Yoshida *et al.* isolated a novel bacterial strain, *Ideonella sakaiensis* 201-F6, from a
151 PET bottle recycling site that is able to both degrade and assimilate highly crystallized PET.²⁶ In
152 contrast to this extraordinary finding and subsequent optimization of the PETase enzyme,⁴⁶ our
153 knowledge on plastic-degrading marine bacteria and their enzymes is very limited and awaits
154 confirmation of the metabolic mechanisms. The limited information available was recently
155 reviewed by Jacquin *et al.*⁷⁸ Whereas biodegradation of PET under aerobic conditions is known
156 to be initiated by the PETase enzyme,²⁶ the most common biodegradation pathway for PS is
157 initiated by a styrene monooxygenase.⁷⁸ For PP and PE such information is not available, but it is
158 generally thought that PE biodegradation is initiated by either biotic or abiotic oxidation of the
159 PE chain, and oxidoreductases, laccases and an alkane hydroxylase have been found to play
160 central roles.^{78,79} Sudhakar *et al.* discovered two marine *Bacillus* strains capable of degrading
161 low-density and high-density polyethylene (LDPE and HDPE) in 2008 (**Table 2**).⁸⁰ However,
162 this degradation happened at very low rates with a maximum weight loss of 10% and 3.5% for
163 LDPE and HDPE, respectively, after 1 year. If the plastic samples were pretreated thermally, the
164 percentage of weight loss increased significantly, indicating that preceding weathering of plastic
165 might make it more accessible for biodegradation by bacteria.⁸⁰ Further marine plastic degraders
166 belonging to the *Bacillus* genus have been isolated from mangrove sediment.⁸¹ A *B. cereus* strain

167 was able to degrade both PE, PET and PS microplastics at rates of 1.6%, 6.6%, and 7.4% weight
 168 loss, respectively, in 40 days. A *B. gottheilii* strain degraded PE, PET, PS, and PP at rates of
 169 6.2%, 3.0%, 5.8%, and 3.6% weight loss, respectively, in 40 days.⁸¹ A third strain, *Bacillus sp.*
 170 27, was able to degrade PP at a rate of 4% in 40 days.⁸² Two other marine Bacilli, *B. pumilus*
 171 M27 and *B. subtilis* H1584, isolated from coastal waters of the Arabian Sea were able to degrade
 172 LDPE at rates of 1.5% and 1.75% in 30 days, respectively.⁸³ Gram-positive plastic-degrading
 173 bacterial isolates include the marine *Rhodococcus sp.* strain 36 (degradation of PP with 6.4%
 174 weight loss in 40 days)⁸² and the *Kocuria palustris* strain M16 (degradation of LDPE with 1%
 175 weight loss in 30 days).⁸³

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

class	species and strain	degraded plastic type	% weight loss	duration of experiment	% weight loss per year	origin of bacterial sample	reference
Actinobacteria	<i>Kocuria palustris</i> M16	LDPE	1	30 days	12.2	Seawater, Arabian Sea	⁸³
	<i>Rhodococcus sp.</i> 36	PP	6.4	40 days	58.4	Mangrove sediment, Malaysia	⁸²
Bacilli	<i>Bacillus cereus</i> BF20	LDPE, HDPE	5, 2	1 year	5.0, 2.0	Indian Ocean	⁸⁰

<i>Bacillus cereus</i>	PE, PET, PS	1.6, 7.4	6.6, 40 days	14.6, 60.2, 67.5	Mangrove sediment, Malaysia	81
<i>Bacillus gottheilii</i>	PE, PET, PP, PS	6.2, 3.6, 5.8	3.0, 40 days	56.6, 27.4, 32.9, 52.9	Mangrove sediment, Malaysia	81
<i>Bacillus pumilus</i> M27	LDPE	1.5	30 days	18.3	Seawater, Arabian Sea	83
<i>Bacillus sp. 27</i>	PP	4	40 days	36.5	Mangrove sediment, Malaysia	82
<i>Bacillus sphaericus</i> Alt	LDPE, HDPE	10, 3.5	1 year	10.0, 3.5	Indian Ocean	80
<i>Bacillus subtilis</i> H1584	LDPE	1.75	30 days	21.3	Seawater, Arabian Sea	83

178

179 Additionally, microbial consortia with plastic-degrading activities have been investigated in
180 the laboratory. A synthetic community of four *Vibrio* strains showed biodegradation of polyvinyl
181 alcohol-low linear density polyethylene (PVA-LLDPE).⁸⁴ Another consortium developed by
182 acclimatizing and bioaugmenting an indigenous community isolated from seawater was able to
183 degrade PE film with 19% weight loss in 6 months.⁶¹ The effect of indigenous microbial
184 consortia on plastic submerged in seawater was detected as percentage weight loss of up to 2.5%

185 in LDPE in six months,⁸⁵ 1.6% in HDPE in 12 months,⁸⁶ 0.6% in PP in six months,⁸⁵ 30% in
186 nylon 4 in 3 weeks,⁸⁷ 0.69% in polycarbonate (PC) in 12 months.⁸⁶ While microorganisms
187 colonizing the plastic surfaces were observed,^{85,86} it should be noted that the respective
188 percentage weight losses cannot be exclusively attributed to biodegradation by these
189 microorganisms. Nevertheless, the findings indicate that the microbial communities colonizing
190 marine plastic litter are of interest in the search for plastic-degrading marine bacteria; however,
191 the identification and characterization of plastic-degrading pathways from the marine
192 environment remains to be awaited.

193 **4. Discussion**

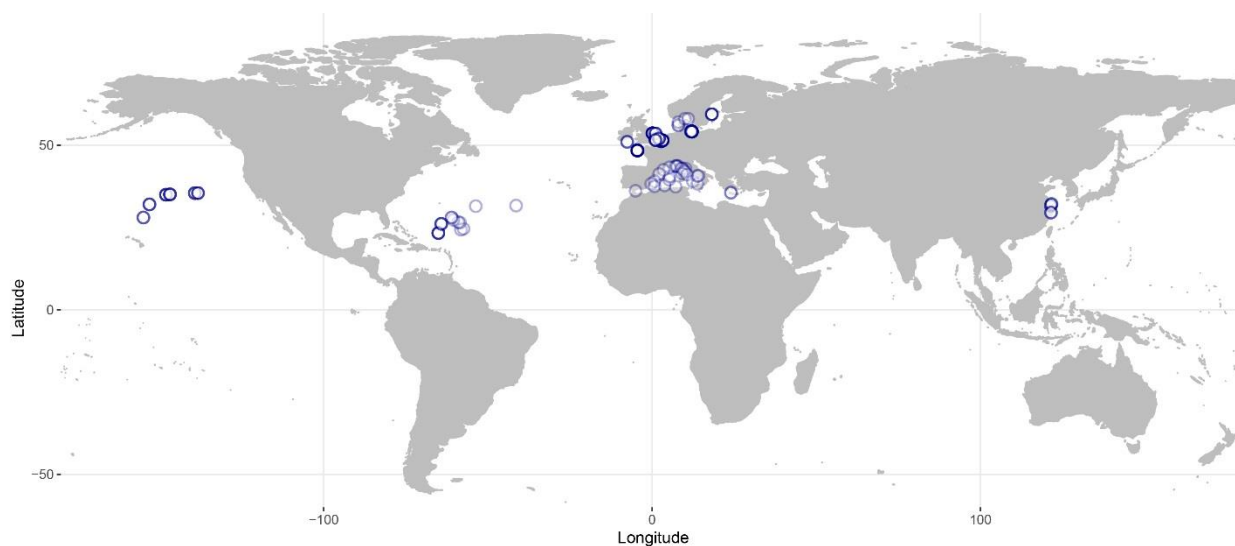
194 Certain bacterial orders and families are repeatedly highlighted in an increasing number of
195 studies on the microbial communities colonizing marine plastic debris, giving rise to the term
196 “plastisphere” for this distinct habitat.³¹ This term has, however, also been challenged due to
197 variability in the plastisphere in regard to geographical and environmental differences^{35–37,58}.
198 Harrison *et al.*⁸⁸ determined the taxonomy of bacteria colonizing LDPE in marine sediment in an
199 estuary and found two main genera; *Colwellia* and *Arcobacter*, which have not been found by
200 studies sampling plastic debris in the pelagic water column. It has been proposed that the core
201 microbiome differs depending on type or age of the plastic substrate, broadening the term
202 “plastisphere” for a specific microbial composition on marine plastic.^{38,59} On the other hand,
203 researchers have demonstrated the significant difference between the microbial communities on
204 marine plastic litter and the surrounding seawater and sediment at different geographical
205 locations such as the North Pacific Subtropical Gyre, the North Atlantic Subtropical Gyre, and
206 the North Sea.^{31,35,38,39} Furthermore, it was shown that bacterial communities attached to plastic
207 particles differed from those attached to a range of marine organic particles³³ as well as cellulose

208 and glass beads.⁴⁰ However, others have found no significant difference in community
209 composition of bacteria colonizing plastic particles and glass beads,³⁵ indicating that the
210 communities might form on any hard surface, not exclusively on plastic surfaces. This is a very
211 important aspect to be addressed in future research within this area. The plastic-associated
212 communities have certain bacterial families in common even across geographical locations
213 (Table 1),³⁹ supporting the idea of a core microbiome native to the “plastisphere”. Furthermore,
214 certain genes or taxon-associated functionalities are commonly enriched in the “plastisphere”.
215 Often, this includes metabolic pathways associated with degradation of xenobiotics,^{39,56,59}
216 indicating that bacteria colonizing plastic surfaces have common genomic traits and could
217 metabolically respond to the plastic, its additives or other adsorbed pollutants. Additionally,
218 some of the most commonly identified bacterial families associated with plastics, such as
219 Rhodobacteraceae and Hyphomonadaceae, also contain hydrocarbon-degrading members or
220 members previously associated with oil spills.³⁹ As stated previously,³⁶ the research community
221 should however be aware of environmental, geographical and surface-dependent differences in
222 the microbial community in future research efforts.

223 **5. Perspectives and Outlook for Marine Plastic Biodegradation**

224 While this perspective attempts to give an overview of a potential core microbiome on marine
225 plastic surfaces as well as marine plastic-degrading bacteria based on the presently available
226 data, it is evident that more data and research are needed. The current knowledge provides
227 indications for a core microbiome in the plastisphere, but in order to understand potential
228 degradation processes and to trace and potentially exploit marine plastic-degrading bacteria,
229 several steps of research are needed:

230 **5.1 Collection of Data.** Currently available data originated from very limited geographic
231 areas in the Northern hemisphere (**Figure 1**). More data from different geographical locations,
232 particularly the continuously exposed plastic-polluted ocean areas such as the major ocean gyres,
233 as well as seasons and environments (sediment vs. pelagic waters) characterizing the unique
234 bacterial communities on plastics are needed. The next-generation sequencing approach of the
235 hypervariable regions of the 16S rRNA gene, first introduced on the plastisphere by Zettler *et*
236 *al.*,³¹ will be sufficient to confirm the core microbiome on plastic. Efforts should be adapted to
237 protocols of the Earth Microbiome Project (<http://www.earthmicrobiome.org/>) to produce
238 consistent data comparable across different experiments and sampling sites.⁸⁹ Furthermore,
239 future studies should collect data on colonization of non-plastic surfaces along with plastic
240 surfaces for comparison of the bacterial communities, similar to the approach of Oberbeckmann
241 *et al.*³⁵ Time series of plastic colonization patterns should be analyzed similar to those conducted
242 on other marine particles such as chitin to elucidate the different roles of primary, secondary etc.
243 colonizers on the material and the overall biofilm structure.⁹⁰ These studies could clarify if the
244 bacteria in the plastisphere actually degrade or modify the material or simply use it as a surface
245 and grow on more readily available photosynthetically-produced carbohydrates. Metagenomic or
246 metatranscriptomic data that not only provide information on taxonomic, but also functional
247 units (present or expressed genes) as described by Bryant *et al.*³⁹ will be important to assess the
248 potential of bacterial colonizers to degrade the plastic and discover novel plastic-degrading
249 pathways.⁹¹ This data can furthermore be utilized to screen for novel homologs of known plastic-
250 degrading enzymes.^{92,93}



251

252 **Figure 1.** World map with sampling sites of currently available biodiversity data on marine
 253 plastic debris (blue rings).^{31,33,35–40,55–59,88}

254 **5.2 Assessment of a Core Plastic-Degrading Microbiome.** Based on sequence data
 255 collected from marine plastic samples, a core microbiome for or core functionalities on marine
 256 plastics should be investigated. This core microbiome might cover general marine plastic litter or
 257 divide into sub-groups by season, geography or plastic type based on the statistical significance
 258 levels of data collected.

259 **5.3 Search for Plastic-Degrading Bacteria or Consortia.** With a core microbiome assessed,
 260 a qualified search for marine plastic-degrading bacteria or consortia can be conducted. This
 261 research will most likely be based on culture-dependent methods or on bioinformatic analyses of
 262 -omic data from marine plastic samples. Ultimately, any bioinformatic results will be followed
 263 up by culture-dependent methods by necessity to establish whether plastic degradation occurs *in*
 264 *vivo*. The core microbiome and associated degradation tests will furthermore indicate if bacteria

265 indeed contribute to the fate of plastic in the marine environment and if natural bioremediation
266 does occur.

267 **5.4 Optimization and Implementation of Plastic-Degrading Bacteria and their Enzymes.**

268 Following the isolation of plastic-degrading bacteria, the enzymes involved in plastic
269 degradation can be characterized, investigated, and optimized as previously shown.^{26,46}
270 Investigation of the evolutionary origin of plastic-degrading enzymes will contribute to the
271 search for other plastic-degrading enzymes and development strategies.^{26,46} Optimization of
272 enzymes through acclimatization experiments, adaptive laboratory evolution and genetic or
273 protein engineering can also be performed to increase efficiency^{46,93,94}. Utilization of plastic-
274 degrading enzymes for bioremediation purposes must go through careful risk assessment and
275 utilization of bacterial strains in the environment should be closely monitored and risk assessed
276 to control spread of their bioactivities. Biofilters entrapping live, plastic-degrading bacteria could
277 however provide an applicable method of bioremediation.⁹⁵ Application of plastic-degrading
278 pathways for the utilization of plastic as a feedstock for cell factories and their most appropriate
279 integration into host metabolism can be guided through metabolic reconstructions of the
280 production hosts⁹⁶ and scaled up in bioreactors.⁹⁷

281 **5.5 Interdisciplinary Work.** A key component of future work on biodegradation of plastic
282 will also be the collaboration of scientists from various disciplines next to microbiologists
283 including, but not limited to: Bioinformaticians and structural biologists to develop *in silico*
284 methods for the reliable identification of putative plastic-degrading enzymes and subsequent
285 precursor/monomer processing; material scientists and chemists to design fluorescently or
286 isotope labelled polymers or polymer stains⁹⁸ to trace biodegradation processes; analytical
287 chemists, ecotoxicologists and oceanographers to provide the analytical and computational

288 techniques to accurately measure and model concentration and distribution of plastic particles in
289 the environment; experts in methods to visually describe the biofilms on the plastic surface and
290 the decay of the polymer itself such as electron, confocal, and atomic force microscopy, Fourier
291 Transform Infrared (FTIR) and Raman spectroscopy.

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

298

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307 **Author Contributions**

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

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313 ABBREVIATIONS

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

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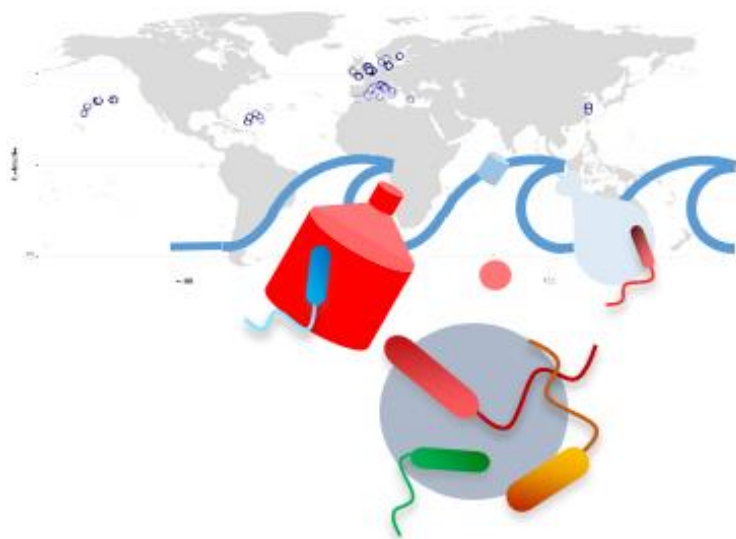
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