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BRIEF COMMUNICATION

Bacterial communities associated with individual transparent exopolymer particles (TEP)

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Transparent exopolymer particles (TEP) are polysaccharide-rich microgels that are prevalent in the marine environment and have important roles in the aggregation of organic matter and carbon export from the euphotic zone. TEP are readily colonized by bacteria and utilized by specialized taxa, such as *Alteromonadaceae*. However, bacterial community composition specifically attached to natural TEP remains largely unknown. In this study, we isolated individual TEP from Plymouth Sound (UK) and performed DNA sequencing of the TEP-attached bacterial communities. We also sampled the cognate bulk seawater total bacterial communities for comparison. The bacterial communities associated with individual TEP showed distinct differences compared to the total bulk bacterioplankton communities, with *Alteromonadaceae* significantly more abundant on TEP. The TEP-associated *Alteromonadaceae* consisted of two operational taxonomic units that were closely related to *Marinobacter* and *Glaciecola*, both previously associated with biogenic aggregates and microgel-rich habitats. This study provides novel insight into marine bacterial–microgel interactions.

KEYWORDS: microbial ecology; bacteria; transparent exopolymer particles; TEP; *Alteromonadaceae*

Transparent exopolymer particles (TEP) are ubiquitous microgels in the marine environment (Passow, 2002a), which are formed by the aggregation of biogenic precursors and maintain a biogeochemical pathway for particu-

late organic matter (POM) formation from dissolved OM (Passow, 2002a; Verdugo *et al.*, 2004). Polysaccharide-rich phytoplankton exudates are a main component of TEP, especially in the upper water column (Passow, 2002b).

Due to their sticky nature, TEP aid in the aggregation of OM and microbes, facilitating carbon export from the euphotic zone and sustaining the biological carbon pump (Engel *et al.*, 2004; Mari *et al.*, 2017). Some TEP are neutrally buoyant (Azetsu-Scott and Passow, 2004) and become enriched at the sea–air interface forming the sea surface microlayer (Cunliffe and Murrell, 2009), where they can potentially contribute to cloud condensation nuclei via bubble bursting into the atmosphere (Quinn and Bates, 2011). TEP are also utilized as a food resource by some zooplankton (Passow, 2002a), such as the copepod *Calanus pacificus* (Ling and Alldredge, 2003).

TEP ecosystem function depends on the lifetime of TEP, including microbial processing. TEP can provide microbes with surfaces for attachment and are readily colonized by marine bacteria (Busch *et al.*, 2017; Engel *et al.*, 2017), which can use TEP either solely as an attachment site or as a carbon source (Passow, 2002a; Taylor and Cunliffe, 2017). Changes in bulk community abundance of *Flavobacteriales*, *Rhodobacterales* and *Alteromonadales* have been correlated with changes in fluctuating seawater TEP concentrations (Taylor *et al.*, 2014; Taylor and Cunliffe, 2017), and *Alteromonadales*, particularly the genus *Alteromonas*, have also been shown to assimilate ¹³C-TEP carbon using DNA stable-isotope probing (Taylor and Cunliffe, 2017).

Even though the importance of TEP in the marine carbon cycle is widely accepted, little is known about the diversity of bacterial communities specifically attached to natural TEP. Based on previous studies, we hypothesize that the bacterial communities on TEP are distinctly different from the surrounding total bulk bacterioplankton community, with the *Alteromonadaceae* particularly associated with natural seawater TEP. To test this hypothesis, we collected and isolated seawater TEP from Plymouth Sound (UK) by staining with Alcian Blue and picking individual TEP using pulled glass pipettes under a microscope. Individual TEP DNA was subsequently used to analyse bacterial communities with high-throughput 16S rRNA gene sequencing and compared with cognate bulk seawater community DNA (see Supplementary Materials and Methods).

Samples were collected from Plymouth Sound (UK) in 3 replicates for the total bulk water community and 30 replicates for TEP microbial communities (i.e. 10 TEP from each of the 3 reciprocal bulk water samples). The isolated TEP differed in size between 184 and 4977 μm^2 (average $964 \pm 1002 \mu\text{m}^2$) (Fig. 1A, Table S1). After vigorous quality control (see Supplementary Materials and Methods), including removing 16S rRNA gene sequences that were found in blanks and operational taxonomic units (OTUs) that appeared

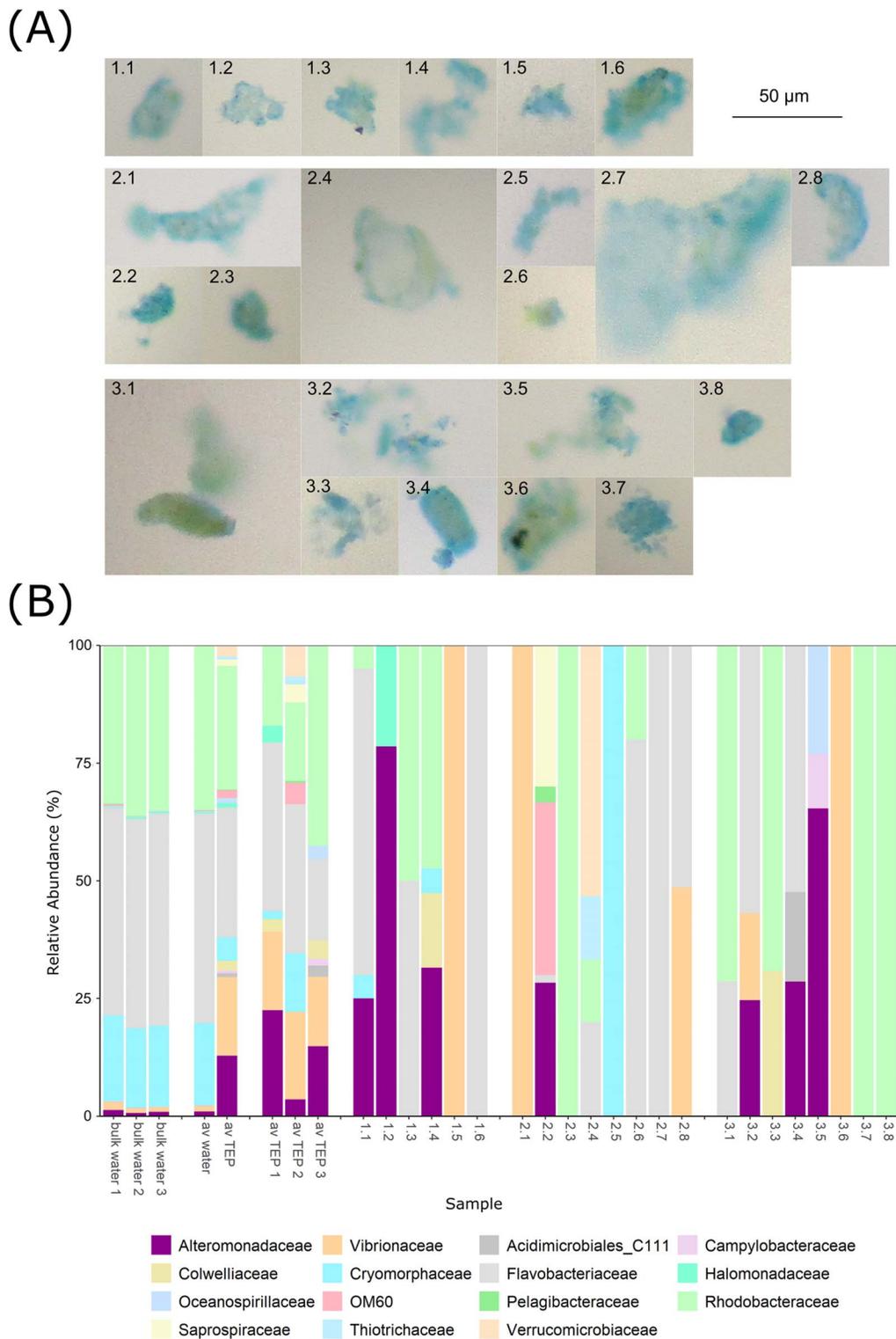
only on single TEP, 22 TEP were used for bacterial community analysis harbouring 26 OTUs (Figs 1B and 2B). The combined TEP-associated bacterial community ($n = 22$) showed clear differences in composition compared to the total bulk water bacterial communities (Fig. 1B). The communities associated with individual TEP were variable, with *Alteromonadaceae*, *Vibrionaceae*, *Rhodobacteraceae* and *Flavobacteriaceae* being the TEP-dominating families (Figs 1B and 2B). *Alteromonadaceae* was significantly increased on TEP compared to the bulk water community (t -test, $P 0.02$) (Fig. 2A), with the closest relatives of the two TEP-associated *Alteromonadaceae* OTUs most similar to *Marinobacter adhaerens* (OTU00071; 93.9% similarity) and *Glaciecola sp.* (OTU00195; 99.3% similarity) (Fig. 2B).

Alteromonadaceae have been shown previously to increase in abundance after phytoplankton blooms in the North Sea (Teeling *et al.*, 2016). *Alteromonadaceae* genera, such as *Alteromonas* and *Glaciecola*, were abundant in the TEP-enriched sea surface microlayer during an induced phytoplankton bloom in a fjord mesocosm experiment (Cunliffe *et al.*, 2009a, 2009b). *Alteromonadaceae* are also physiologically well equipped for the degradation of phytoplankton exudates, including the production of a diverse range of carbohydrate-active enzymes (Teeling *et al.*, 2016).

In this study, *Alteromonadaceae* were composed of two OTUs. OTU00071 was most closely related to several *Marinobacter* species, such as *M. adhaerens*, which was originally isolated from a marine aggregate formed by the diatom *Thalassiosira weissflogii* (Kaeppl *et al.*, 2011). OTU00195 was closely related to several *Glaciecola* species. In a recent study, *Glaciecola* were shown to dominate the bacterial community during early diatom blooms and were suggested to be the main consumer of phytoplankton-derived OM during early bloom stages (von Scheibner *et al.*, 2017).

The size range of TEP isolated during this study was focused towards TEP larger than 184 μm^2 due to feasibility of handling TEP with pulled glass pipettes under a microscope. While this study does not consider small TEP, previous studies found that smaller particles are less densely colonized by bacteria than larger particles (Schuster and Herndl, 1995; Passow, 2002a), suggesting that bacterial degradation might be more important for larger TEP.

Our study presents a new perspective on studying individual natural TEP for molecular microbial ecology analyses. To the authors' knowledge, this is the first direct detection of *Alteromonadaceae* being enriched on natural TEP. Their enrichment, together with previous studies showing *Alteromonadaceae* are able to assimilate TEP-derived carbon (Taylor and Cunliffe, 2017)



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Fig. 1. (A) Microscopy pictures of the 22 isolated TEP with scale bar (since TEP were photographed in seawater without cover slip, some TEP seem to contain gaps due to the limited depth of the focal plane). (B) Bacterial community composition on TEP and in bulk water. Bulk water replicates 1–3 and bulk water average ($n = 3$). Combined TEP average ($n = 22$). Averages from TEP from the three sampling bottles deployed (TEP1, $n = 6$; TEP2, $n = 8$; TEP3, $n = 8$). All individual TEP are shown.

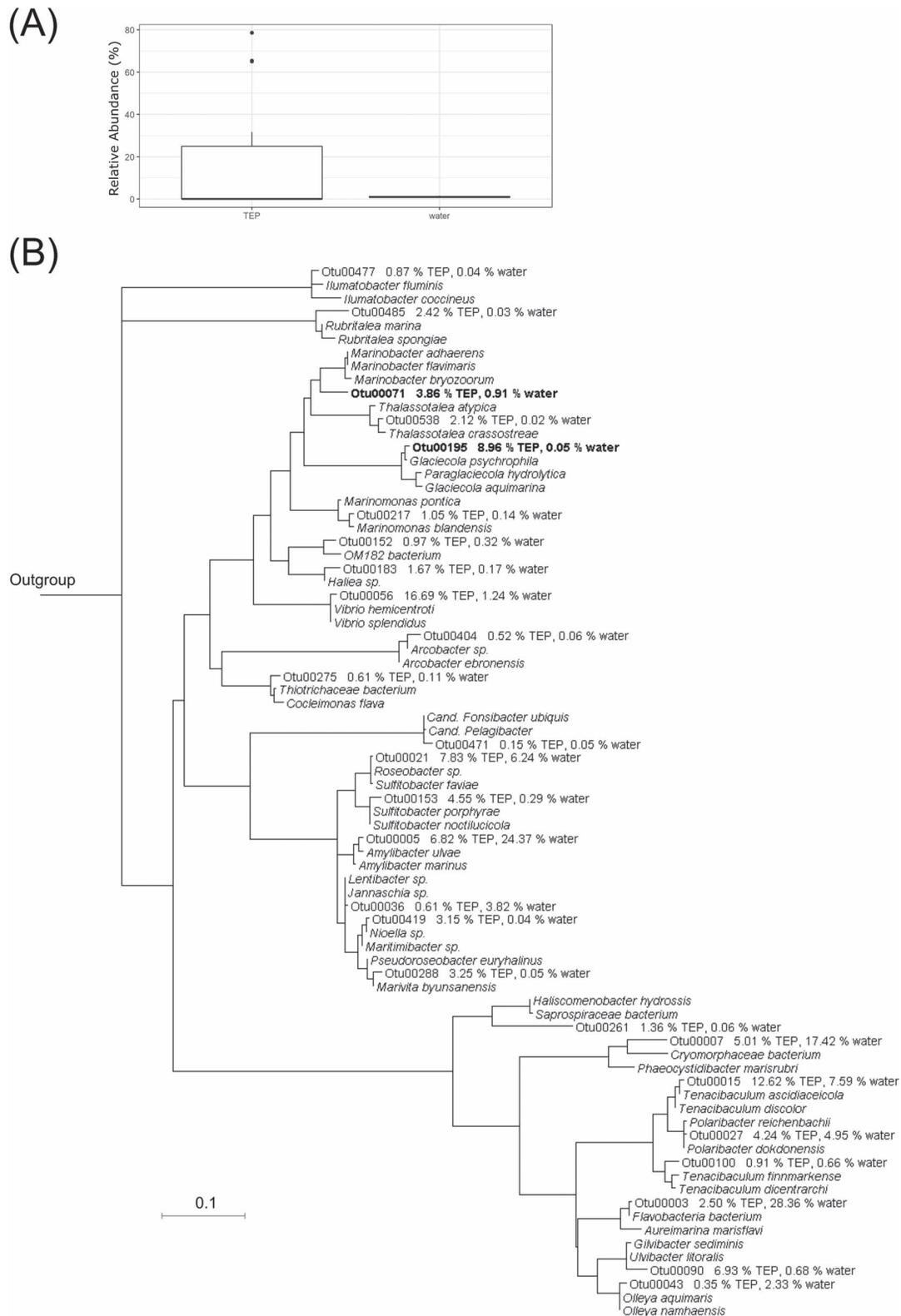


Fig. 2. (A) Relative abundance of the *Alteromonadaceae* OTUs combined on TEP and in the surrounding bulk water. (B) Phylogenetic tree depicting the closest relatives of all OTUs found on TEP. The outgroup used was *Chlamydia sp.* Sequences were aligned with MULTiple Sequence Comparison by Log- Expectation and computed using the maximum likelihood algorithm. The average relative abundance of each OTU on TEP and in the bulk water is shown.

and a general association with phytoplankton and phytoplankton-derived OM (Sarmiento and Gasol, 2012; Teeling *et al.*, 2016), enforces the paradigm that they play a role in marine TEP degradation and subsequent aggregation dynamics of OM, which could influence carbon export from the euphotic zone.

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