Sweet spheres: succession and CAZyme expression of marine bacterial communities colonizing a mix of alginate and pectin particles

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Summary
Polysaccharide particles are important substrates and microhabitats for marine bacteria. However, substrate-specific bacterial dynamics in mixtures of particle types with different polysaccharide composition, as likely occurring in natural habitats, are undescribed. Here, we studied the composition, functional diversity and gene expression of marine bacterial communities colonizing a mix of alginate and pectin particles. Amplicon, metagenome and metatranscriptome sequencing revealed that communities on alginate and pectin particles significantly differed from their free-living counterparts. Unexpectedly, microbial dynamics on alginate and pectin particles were similar, with predominance of amplicon sequence variants (ASVs) from Tenacibaculum, Colwellia, Psychrobium and Psychromonas. Corresponding metagenome-assembled genomes (MAGs) expressed diverse alginate lyases, several colocalized in polysaccharide utilization loci. Only a single, low-abundant MAG showed elevated transcript abundances of pectin-degrading enzymes. One specific Glaciecola ASV dominated the free-living fraction, possibly persisting on particle-derived oligomers through different glycoside hydrolases. Elevated ammonium uptake and metabolism signified nitrogen as an important factor for degrading carbon-rich particles, whereas elevated methylcitrate and glyoxylate cycles suggested nutrient limitation in surrounding waters. The bacterial preference for alginate, whereas pectin primarily served as colonization scaffold, illuminates substrate-driven dynamics within mixed polysaccharide pools. These insights expand our understanding of bacterial niche specialization and the biological carbon pump in macroalgae-rich habitats.

Introduction
Polysaccharides produced by marine macroalgae and phytoplankton are important ecological and biogeochemical agents, serving as structural and storage components for the algae as well as nutrient source for heterotrophic bacteria (Amosti et al., 2021). A considerable fraction of algal polysaccharides is bound in particles, hotspots of microbial activity with central implications for the biological carbon pump (Stocker, 2012). Hydrogels and transparent exopolymer particles (TEP), a subset of polysaccharide particles forming by self-assembly of anionic polysaccharides in seawater, constitute a global amount of ~70 gigatons and are indispensable for the study of particle–microbe interactions (Verdugo et al., 2004; Verdugo, 2012; Cordero and Datta, 2016). The building blocks of marine hydrogels largely originate from macroalgae, in which anionic gelling polysaccharides such as alginate can constitute >50% of the biomass (Mabeau and Kloareg, 1987). Natural processes of decay or exudation, such as the release of alginate and rhamnogalacturonan from widespread macroalgae (Koch et al., 2019a), presumably result in the formation of hydrogel scaffolds that represent hotspots for microbial life. These events potentially play an ecological role at rocky coasts of temperate seas, which harbor dense forests of macroalgae.

The chemical and structural complexity of marine hydrogels challenges the identification of specific
particles compared to the surrounding water (Mitulla et al., 2016; Sperling et al., 2017; Zäncker et al., 2019). Furthermore, attached microbes can undergo a temporal succession of primary degraders and opportunistic taxa (Datta et al., 2016; Enke et al., 2018, 2019). The main indicator of hydrolytic capacities is the presence and diversity of carbohydrate-active enzymes (CAZymes), foremost polysaccharide lyases (PLs) and glycoside hydrolases (GHs), in bacterial genomes (Hehemann et al., 2014). CAZymes are commonly clustered in polysaccharide utilization loci (PUL), operon-like regions facilitating efficient hydrolysis (Grondin et al., 2017). CAZyme numbers, diversity and genomic organization can distinguish bacteria in primary degraders for initial polymer breakdown, and secondary consumers utilizing oligosaccharides, monosaccharides or other compounds released by primary degraders. These types occur across taxonomic boundaries and also within single species (Hehemann et al., 2016; Koch et al., 2020).

Nonetheless, it remains enigmatic how bacterial particle utilization proceeds within the natural ‘particlescape’ – presumably containing a mixture of particle types with different polysaccharide composition – and how these processes are shaped by the taxonomic and functional diversity of the ambient microbiota. The co-availability of hydrogels with different polysaccharide composition might initiate a segregation of bacterial populations by substrate preferences, comparable to hydrolyzing model isolates (Zhu et al., 2016; Koch et al., 2019a). In this context, the CAZyme repertoire is considered to be a stronger driver of niche specialization than phylogenetic relationships (Hehemann et al., 2016; Wolter et al., 2021a). The colonization and utilization of particle resources might also include interactions with free-living microbes, which might benefit from oligosaccharides and other compounds released into the surrounding water. In addition, microbial competition and cooperation can coincide with successional patterns and specific interactions (Ebrahimi et al., 2019; Gralka et al., 2020).

To evaluate particle-specific bacterial dynamics in a mixture of hydrogels, the present study co-exposed alginates and pectin particles to bacterioplankton communities from Helgoland, an island in the southern North Sea surrounded by dense macroalgal forests (Bartsch and Kuhlenkamp, 2000; Uhl et al., 2016). Due to the gelling capacities of alginates and pectin and their demonstrated release from Helgoland macroalgae (Koch et al., 2019a), we assume that related particles occur in this habitat and constitute microhabitats for specialized microbiota. The co-incubation followed by magnetic separation allowed deciphering community composition, functional potential and gene expression depending on particle type and in relation to the free-living fraction. Opposed to our original hypothesis that alginates and pectin particles are utilized by different members of the ambient community, we observed similar compositional and functional patterns with predominant expression of alginase lyases. The identification of alginates as preferred substrate, whereas pectin primarily served as colonization scaffold, illuminates bacterial microhabitat ecology and substrate cycling in macroalgae-rich habitats with diverse polysaccharide budgets.

Results and discussion

We studied taxonomic diversity, functional capacities and gene expression of particle-attached (PA) marine bacterial communities on alginates (AlgP) and pectin (PecP) particles in comparison to their free-living (FL) counterparts. For this purpose, synthetic AlgP and PecP were co-exposed to bacterioplankton collected near Helgoland Island, surrounded by dense macroalgal forests and hence considerable polysaccharide budgets (Supplementary Fig. 1A). The ambient water was sequentially filtered through 100 and 20 μm before AlgP/PecP addition to exclude naturally occurring particles and larger organisms. For the targeted separation of communities, we then carried out triplicate co-incubations in different combinations of magnetic and non-magnetic particles: (i) magnetic AlgP and non-magnetic PecP, (ii) non-magnetic AlgP and magnetic PecP, and (iii) controls without particles. Applying magnetic force allowed the specific recovery of each particle type (Supplementary Video 1). The FL fraction was obtained by 5 μm filtration to remove non-magnetic particles and collecting the flow-through on 0.2 μm filters (Supplementary Fig. 1B).

Do AlgP, PecP and FL harbour specific communities with temporal variability?

Amplon sequencing of bacterial 16S rRNA genes revealed significant differences between PA and FL communities (PERMANOVA, p < 0.001) but substantial overlap between AlgP and PecP (Fig. 1A, Supplementary Fig. 2A). FL communities from both particle combinations were congruent as expected (Fig. 1A), and FL data were thus combined in subsequent analyses. Furthermore, significant compositional differences of PA and FL communities to those in the ambient seawater and controls without added particles (PERMANOVA, p < 0.001) confirmed the observations as true biological dynamics.
Amplicon sequence variants (ASVs) affiliated with *Tenacibaculum* (*Bacteroidetes: Flavobacteriales*), *Colwellia*, *Psychromonas* and *Psychrobium* (*Gammaproteobacteria: Alteromonadales*) constituted up to 60% of both AlgP and PecP communities (Fig. 1B), with significant enrichment compared to the FL fraction (Kruskal-Wallis test, \( p < 0.001 \)). Hence, particle colonization largely related to few dominant taxa, comparable to other marine polysaccharide particles (Datta et al., 2016; Enke et al., 2019). The finding of related strains with considerable CAZyme repertoires on marine macroalgae (Dong et al., 2012; Martin et al., 2015; Gobet et al., 2018; Christiansen et al., 2020) supports the ecological relevance of our observations. Notably, both *Colwellia* and *Tenacibaculum* can be enriched on decaying algae (Fernandes et al., 2012; Zhu et al., 2017) and hence under circumstances when algal polysaccharides might be released and self-assemble into particles. Furthermore, *Tenacibaculum* and *Psychromonas* frequently occur during phytoplankton blooms near Helgoland, when bacterial dynamics are largely driven by algal carbohydrates (Teeling et al., 2012; Kappelmann et al., 2019; Krüger et al., 2019). High adaptability and metabolic rates, illustrated by the rapid stimulation of multiple *Colwellia* ASVs from nearly undetectable levels in the ambient community (Supplementary Fig. 3), could be a competitive advantage during such events.

(*Glaciecola* (*Alteromonadales*)) dominated the FL community (Kruskal–Wallis test; \( p < 0.0003 \)), with an average abundance of >30% during the first 48 h with low alpha-diversity (Fig. 1B, Supplementary Fig. 2B). Notably, the *Glaciecola* population was dominated by a single ASV (Supplementary Fig. 3), suggesting that nutrient scarcity in FL favoured highly competitive genotypes. This finding underlined that specific biogeochemical conditions can...
stimulate the predominance of single community members (Pedler et al., 2014). We hypothesize that *Glaciecola* largely persisted as secondary consumer of particle-derived substrates, supported by genomic evidence from the major corresponding MAG (see below).

The substantial overlap between AlgP and PecP microbiomes contradicted our original hypothesis that the ambient community segregates by particle type. Furthermore, there was little temporal variability in community composition, although we possibly missed rapid sequential dynamics as observed in related studies (Datta et al., 2016; Enke et al., 2019). One exception was *Pseudoalteromonas*, whose sole occurrence at 24 h on both particle types (Fig. 1B; Kruskal–Wallis test, \( p = 0.002 \)) signifies a polysaccharide pioneer (Hehemann et al., 2016). This notion is supported by alginolytic and pectinolytic capacities of various *Pseudoalteromonas* species, which generally respond quickly to nutrient input (Ivanova et al., 2014; Hehemann et al., 2017). The AlgP microbiota established within the first 24 h and then remained unchanged, whereas *Tenacibaculum* established with temporal delay on PecP with peak abundances at 60 h (Fig. 1B; Kruskal–Wallis test, \( p = 0.04 \)). Faster stabilization of the AlgP community indicates that alginate was the major nutrient source, as discussed below in context of metagenomic and metatranscriptomic evidence. One notable exception was *Carsonovulum* (*Alteromonadales*), which solely established on PecP after 60 h (Fig. 1B; Kruskal–Wallis test, \( p = 0.01 \)) and was the only taxon linked to pectin degradation (see below).

**Do AlgP, PecP and FL communities differ in functional diversity and gene expression?**

As taxonomic and metagenomic richness are overall connected (Salazar et al., 2019), we expected contrasting functional potentials in PA and FL communities, whereas metabolic capacities of AlgP and PecP communities should be largely congruent. However, AlgP and PecP microbiomes might differ in gene expression patterns, as these can be independent from taxonomic composition (Salazar et al., 2019). For instance, certain taxa encode both alginate and pectate lyases (Koch et al., 2019a) and might express the corresponding genes differently depending on particle type. To evaluate these aspects, we analyzed the metagenome (24 and 60 h) and metatranscriptome (60 h) of AlgP and PecP communities in relation to the FL fraction (Supplementary Table 1). This approach included both community-wide and genome-centric perspectives through MAGs.

The metagenomic library of 21 gigabases comprised \( \sim 192 \) 000 genes predicted by Prokka, 47% of which were functionally annotated using UniProtKB, KEGG and/or COG databases. Two percent of all genes were predicted to encode CAZymes according to dbCAN2 (Supplementary Table 2). We first assessed overarching differences between PA (i.e. occurring on both AlgP and PecP) and FL communities to identify general signatures of planktonic and attached niches. Transcripts from the citric acid cycle, glycolysis/gluconeogenesis and amino acid metabolism were abundant in both PA and FL metatranscriptomes but numerous pathways differed (Supplementary Table 3). Overall, \( \sim 60\% \) of all transcripts were differentially abundant between PA and FL communities (Fig. 2A, Supplementary Table 3), matching metatranscriptomic evidence in other marine ecosystems (Satinsky et al., 2014). Transcript abundances of glutamine synthetase, one key enzyme of bacterial nitrogen assimilation converting ammonium into glutamine, peaked in PA communities (Fig. 2B). This observation suggests considerable ammonium uptake to meet the nitrogen demand for fuelling polysaccharide-derived carbon into protein biosynthesis, supported by abundant transcripts of related transport and regulator genes (Fig. 2B; Wilcoxon rank-sum test, \( p < 0.05 \)). Notably, the biosynthesis of valine, leucine and isoleucine peaked in PA, but their degradation in FL communities (Supplementary Table 3; Wilcoxon rank-sum test, \( p < 0.01 \)). We interpret this observation as provision of amino acids from actively growing PA to substrate-limited FL bacteria. In this context, leucine exchange between bacteria on polysaccharide particles and the surrounding water (Enke et al., 2019) might be a stabilizing component of their interactions (Johnson et al., 2020). Glyoxylate, dicarboxylate and pyruvate metabolism peaked in FL communities (Supplementary Table 3). Furthermore, induction of the methylcitrate cycle and the glyoxylate shunt (Fig. 2B; Wilcoxon rank-sum test, \( p < 0.01 \)) supports the notion of substrate limitation in the FL niche, matching transcriptomic responses of starved bacterioplankton (Kaberdin et al., 2015). These pathways likely promoted persistence by generating energy from short-chain fatty acids but might also alleviate iron limitation or oxidative stress (Palovaara et al., 2014; Ahn et al., 2016; Dolan et al., 2018; Koedooder et al., 2018; Serafini et al., 2019). In *Alteromonas macleodii*, similar expression patterns were interpreted as maintenance metabolism (van Bodegom, 2007; Beste and McFadden, 2010; Koch et al., 2019b).

Next, we specifically compared AlgP and PecP to identify polysaccharide-specific patterns. Communities on AlgP and PecP only slightly differed in functional potential and gene expression (Fig. 2A), compliant with their compositional overlap (Fig. 1). Only 2% of transcripts were differentially abundant, without community-wide patterns
in specific functional categories (Supplementary Table 3). On AlgP, higher transcript abundances of alkaline phosphatase genes possibly counteracted beginning phosphate limitation, comparable to late stages of natural TEP colonization (Berman-Frank et al., 2016). Furthermore, higher transcript abundances of predicted prophages (Fig. 2C, Supplementary Table 3) indicates the induction of lytic cycles and corresponding release of organic matter (Breitbart et al., 2018). These events potentially stimulated secondary consumers such as *Aureispira*, which only appeared after 60 h (Kruskal–Wallis test, \( p = 0.01 \)). This predatory taxon can feed on metabolic products or cell debris from other bacteria, fuelled by its capacity to adhere to anionic polysaccharides (Furusawa et al., 2015). On PecP, a single MAG related to *Catenovulum* accounted for the vast majority of differentially abundant transcripts, supporting the predisposition of this taxon towards pectin (see below). The PecP-specific upregulation of lipopolysaccharide-related *mla*, *ipt* and *kds* genes presumably stimulated biofilm formation, an important advantage for colonization and assimilation of particulate substrates (Sivadon et al., 2019).

**Community-level diversity and expression of CAZymes**

Similarities between AlgP and PecP extended to comparable CAZyme profiles, dominated by PL6 and PL7 alginate lyase genes on both particle types (Supplementary Table 2). PL7 genes for the initial depolymerization of alginate, approximately half including a CBM32 carbohydrate-binding domain, peaked in both copy numbers and transcript abundances (Fig. 3A, Supplementary Table 2). PL15, PL17 and PL18 genes encoding the processing of released oligomers were less numerous but considerably transcribed (e.g. locus tags 183566 and 114168), with the highest transcript abundance of all CAZymes in a PL18 gene (locus tag 127388). The alginate content of >50% in brown macroalgae like
Saccharina and Fucus, which are abundant in our sampling area and release alginate into the water column (Koch et al., 2019a), offers an explanation why alginate-degrading genes and organisms predominated. In contrast, we only detected three PL1 pectate lyases and few other pectin-related genes (CE8, GH28, GH105). These results indicate that pectin is not a prime bacterial substrate in kelp forests, although pectinolytic bacteria occur in diverse marine habitats (Van Truong et al., 2001; Hehemann et al., 2017; Hobbs et al., 2019) and pectinous substrates are exuded by Helgoland macroalgae (Koch et al., 2019a). Instead, we hypothesise that PecP primarily served as colonization scaffolds for alginolytic bacteria. We propose that the predominant taxa are generally adapted to life on (polysaccharide) particles, favoring cross-particle colonization especially as AlgP were available nearby. The fast sinking of the relatively large particles (diameter ~200 μm) resulted in a loose bottom.
layer, with close spatial contact of both particle types. This ‘particlescape’ potentially allowed cross-particle interactions and utilization of alginate, even if attached to PecP. Nonetheless, significantly higher abundances of alginate lyase transcripts on AlgP (Wilcoxon rank-sum test, \( p = 0.0002 \) to \( 10^{-16} \)) indicates that PecP associates were less alginolytic, possibly attributed to diffusion losses.

The FL community showed a completely different CAZyme signature, with elevated transcript abundances of GHs (Fig. 3A and B). Predominance of families GH3, GH13, GH16 and GH23 (Fig. 3A) matches the hydrolase repertoire of FL bacteria during phytoplankton blooms around Helgoland (Teeling et al., 2016; Kappelmann et al., 2019). Hence, our observations resemble responses of natural bacterioplankton to oligosaccharide mixtures. Although these GH families are mainly associated with \( \alpha \)-1,4-glucan, \( \beta \)-1,3-glucan or peptidoglycan degradation (Lombard et al., 2014), our observations indicate a broader range including oligomers of anionic polysaccharides.

**CAZymes and PUL on genomic level**

Analysis of five near-complete MAGs (>90% completeness at <5% contamination) supported the genomic and ecological differentiation between PA and FL communities (Table 1, Supplementary Table 4). Core-gene phylogeny demonstrated that these MAGs represent the major PA and FL members *Colwellia*, *Tenacibaculum*, *Psychromonas* and *Glaciecola* (Fig. 4A, Supplementary Fig. 4). Accordingly, their normalized coverage matched amplicon-based abundances (Fig. 4B).

Approximately 3% of genes in the major PA-MAGs were annotated as CAZymes (Table 1), mostly PL6, PL7 and PL18 alginate lyases with CBM32, CBM16 or CBM6 domains (Fig. 5). Considerable transcript abundances of alginate lyase and monomer-processing genes *kdgA*, *kdgF*, *kdgK* and *dehR* illustrate the complete metabolization of alginate (Fig. 5, Supplementary Table 4, Supplementary Fig. 5). Approximately half of CAZymes from *Colwellia*, *Psychrobium* and *Psychromonas* MAGs harbour predicted signal peptides (Supplementary Table 4) and were hence likely secreted, although we cannot discern whether these were indeed free enzymes or anchored to the cell membrane. Presumably, CAZyme secretion into the polysaccharide matrix facilitated particle utilization, enhancing polymer hydrolysis and subsequent oligomer uptake (Vetter et al., 1998). MAG Gla-32 affiliated with the dominant FL taxon *Glaciecola* encoded only three PLs but 18 GHs, with the highest transcript abundances of families GH3, GH13 and GH23 (Fig. 5).

The lower fraction of signal peptides in its CAZymes (30%) indicates that secreted enzymes are less relevant when free-living, pointing towards opportunistic interactions with primary hydrolyzers.

Overall, only some CAZymes of each MAG’s repertoire showed elevated transcript abundances (Fig. 5). We assume that the ‘silent’ CAZymes enable the degradation of other carbohydrates. For instance, the *Colwellia*-MAG Col-24 encodes a homolog of the rarely described PL29 family (locus tag 50313), potentially activated in presence of chondroitin sulfate, dermatan sulfate or hyaluronic acid (Ndeh et al., 2018). Although Col-24 clusters with the hydrolytic model isolate *Colwellia echini* A3 (Fig. 4A) at 80% average nucleotide identity (ANI), a BLASTp survey revealed that CAZymes targeting agar, carrageenan and furcellaran are not shared with strain A3 (Supplementary Table 4). Divergent CAZyme repertoires in related *Colwellia* spp. presumably reflect their different habitats (Christiansen et al., 2020).

**MAG-specific polysaccharide utilization loci.** We detected several PUL in the *Tenacibaculum*-MAG Ten-26. For instance, one PUL encodes PL12 and PL17 alginate lyase plus SusCD transporter genes, the hallmark of flavobacterial PUL (Fig. 6A, Supplementary Fig. 6). In contrast, CAZyme genes in gammaproteobacterial MAGs were largely scattered throughout the genomes, although PUL-like operons occur in related taxa (Neumann et al., 2015; Schultz-Johansen et al., 2018; Christiansen et al., 2020). The *Psychromonas*-MAG Psym-73 encodes

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**Table 1.** Characteristics of near-complete metagenome-assembled genomes.

| MAG    | Taxonomy (GTDB-tk)                  | Completeness | Contamination | Size (Mbp) | Genes | CAZymes | PLs/GHs |
|--------|-------------------------------------|--------------|---------------|------------|-------|---------|---------|
| Col-24 | Alteromonadaceae; *Colwellia*       | 90           | 3.0           | 3.27       | 2886  | 91      | 16/18   |
| Ten-26 | Flavobacteriaceae; *Tenacibaculum*  | 97           | 2.0           | 3.06       | 2781  | 65      | 17/9    |
| Gla-32 | Alteromonadaceae; *Glaciecola*      | 96           | 0.6           | 2.77       | 2575  | 49      | 3/18    |
| Psyb-57| Psychrobacteriaceae; *Psychrobium*  | 95           | 0.9           | 2.99       | 2690  | 61      | 16/10   |
| Psym-73| Psychromonadaceae; *Psychromonas*   | 94           | 1.0           | 3.69       | 3337  | 110     | 22/34   |

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A

Psychromonas sp. B3M02
Psychromonas spp.
Psychromonas sp. RZ22
Psychromonas sp. RZ5
Psym-73 (PA)

Col-24 (PA)
Colwellia echini A3
Colwellia spp.
Colwellia sp. RSH04
Colwellia sp.
Glaciecola sp. 4H-3-7+YE-5
Glaciecola spp.
Glaciecola sp. UBA983
Gla-32 (FL)

Tenacibaculum spp.
Tenacibaculum adriaticum\textsuperscript{T}
Tenacibaculum MAR-2010-89
Tenacibaculum sp. E3R01
Tenacibaculum spp.
Tenacibaculum spp.

B

| AlgP | FL |
|------|----|
| Col-24 | 5 |
| Ten-26 | 15 |
| Psyb-57 | 20 |
| Psym-73 | 25 |
| Gla-32 | 30 |

24h  | 60h

Fig 4. Phylogeny and abundance of metagenome-assembled genomes (MAGs).
A. Maximum-likelihood phylogeny based on 92 single-copy core genes in the context of related genomes. Dots designate nodes with >90% bootstrap support. Supplementary Fig. 4 shows an extended tree including medium-quality MAGs and additional related genomes.
B. Normalized coverage in metagenomes at 24 and 60 h. The scales of y-axes differ for better visualization.

Two PL7 from subfamilies 5 and 3, each harboring a CBM16 and CBM32 domain (Fig. 6B, Supplementary Table 4). Together with the adjacent CBM16 gene, this combination indicates efficient binding and processing of different alginate architectures (Sim et al., 2017; Hu et al., 2019). The Psychrobium-MAG Psyb-57 encodes a PL12, a candidate novel variant of alginate lyases shared with other bacteria from Helgoland (Kappelmann et al., 2019). Colocalization of this PL12 with exopolysaccharide-related genes (Fig. 6C) might link polysaccharide degradation and biosynthesis, considering the regulation of exopolysaccharide metabolism via PLs (Bakkevig et al., 2005; Köseoğlu et al., 2015). A comparable gene arrangement in an alginolytic Maribacter strain from the south Atlantic (Wolter et al., 2021b) supports the potential implications for particle colonization.

A GH108 gene unique to Glaciecola-MAG Gla-32, colocalized with GH1 and carbohydrate transporter genes (Fig. 6D), might allow scavenging oligomers released from particles. Gla-32 is related to the deep-sea isolate Glaciecola sp. 4H-3-7 + YE-5 (Fig. 4A), and their share of 40 CAZymes including a GH13 pair and adjacent GH77 (locus tags 06167–06169) indicates ecological relevance in diverse habitats (Klippel et al., 2011). We detected transcripts of PL6 and PL7 lyases as well as the monomer processing pathway in Gla-32 (Fig. 5, Supplementary Table 4), indicating a general ability for alginate depolymerization. However, its incomplete alginate operon compared to known alginate degraders (Supplementary Fig. 7A) might signify inefficient alginolytic activity that limits particle colonization.

Diversity of PL7 homologues in Psym-73. The presence of 14 PL7 genes in the Psychromonas-MAG Psym-73 signifies a marked specialization towards alginate, as...
hydrolytic activity scales with CAZyme number (Hehemann et al., 2016). Two of these homologs (locus tags 20477 and 38641) exhibited the highest transcript abundances of all PL7 genes in our dataset (Supplementary Table 4). Both are related to biochemically characterized lyases from Vibrio strains (Supplementary Fig. 8) isolated from macroalgae or seawater (Roux et al., 2009; Badur et al., 2015; Sun et al., 2019). For instance, PL7_38641 has 72% amino acid identity to AlyD of Vibrio splendidus, an endolytic lyase releasing three oligomer fractions from guluronate-rich sections (Badur et al., 2015). Prediction of a Lipo signal peptide while lacking a CBM indicates that PL7_38641 is anchored as outer membrane lipoprotein (Supplementary Table 4), comparable to AlyA5 from Zobellia galactanivorans (Thomas et al., 2013).

The two adjacent PL7 genes from different subfamilies (Fig. 6B) possess predicted Sec and Lipo signal peptides respectively (Supplementary Table 4), indicating complementary membrane-bound versus secreted localization to maximize alginate utilization. Homologues of PL7_75913 with >50% amino acid identity also occur in Simidua (Cellvibrionales), Reichenbachiella and Marinoscillum (Cytophagales), indicating wide ecological relevance (Spring et al., 2015). PL7_75915 from the poorly described subfamily 3 has 55% amino acid identity to a structurally resolved lyase from Persicobacter (Sphingobacteriales) specialized towards alginate of high molecular weight (Sim et al., 2017), suggesting a role in initial depolymerization.

We hypothesize that the two PL variants originate from separate horizontal acquisition events with subsequent insertion into the same genomic locus, considering their low similarity and different branching in the phylogenetic tree (Supplementary Fig. 8). Overall, highly variable transcript abundances of PL7 genes (Supplementary Fig. 8) suggest that different variants are activated by specific biochemical conditions, for instance, different alginate characteristics (e.g. polymer length; dissolved or particulate form; or the ratio of mannurionate to guluronate monomers).

**Taxa with recurrent occurrence on alginate particles.** We compared MAGs Psym-73 and Ten-26 with the genomes of Psychromonas sp. BSM02 and Tenacibaculum sp. E3R01 respectively; strains isolated in a comparable study on alginate particles (Enke et al., 2019). Supported by ~80% ANI and core-gene phylogeny (Fig. 4A), these
Fig 6. Structure and expression of PUL in MAGs.
A. PUL encoding SusCD and a PL12-PL17 gene pair in Tenacibaculum-MAG Ten-26.
B. PL7 genes from different subfamilies colocalized with a CBM16 gene in Psychromonas-MAG Psym-73.
C. PL12 and exopolysaccharide-related genes (green) in Psychrobium-MAG Psyb-57.
D. Unique GH108 adjacent to GH1 and carbohydrate transporter genes in Glaciecola-MAG Gla-32. Supplementary Table 4 and Supplementary Fig. 6 show detailed gene annotations and PUL architectures. EPS: exopolysaccharide; CHO: carbohydrate.
represent related species with presumably wide ecological relevance on polysaccharide particles. Psym-73 and strain B3M02 share nine homologous PLs (Supplementary Table 5), however, encoded in different genomic contexts. This variable organization, together with the higher PL count in Psym-73, indicates considerable CAZyme diversity and genomic rearrangements among hydrolytic Psychromonas. Ten-26 and strain E3R01 share 29 homologous CAZymes. However, no PLs were detected in E3R01 while Ten-26 encodes 17 (Supplementary Table 5). These observations indicate CAZyme-related niche specialization among Tenacibaculum species, consistent with CAZyme variability in Tenacibaculum type strains ranging from eight PLs in T. jejunese to none in T. mesophilum (Lombard et al., 2014).

A single, rare pectin degrader. Despite the compelling evidence that alginate was the preferred bacterial substrate, MAG21 is a candidate for pectin utilization. MAG21 accounted for ~95% of differentially abundant transcripts on PecP (Fig. 7A), contributing to significantly elevated GH abundances and normalized coverage compared to AlgP (Fig. 7B, Kruskal–Wallis test, p < 10^{-16}). MAG21 encodes several genes for galacturonate degradation and processing of pectin monomers. A GH53 endo-galactanase gene with the fourth-highest transcript abundance on PecP (Fig. 7C) we propose that MAG21 is taxonomically and functionally related to GH28 and GH105 enzymes, which are also encoded by MAG21 (Supplementary Table 4). Together with the restriction of Catenovulum ASVs to PecP (Fig. 1C), we propose that MAG21 is taxonomically and functionally related to Catenovulum and degrades galacturonate. The medium quality of MAG21 (76% estimated completeness) may explain why pectate lyases are missing compared to CCB-QB4. Alternatively, MAG21 indeed only encodes an incomplete degradation cascade, only accessing oligomeric side chains or oligomers released by the activity of primary degraders that encode pectate lyases.

An additional PecP-specific pattern occurred in Psychromonas-MAG Psym-73, with significantly higher transcript abundances of a hybrid gene cluster for the biosynthesis of a siderophore as well as spermidine (Supplementary Table 3, Supplementary Fig. 7B). Homologues of the siderophore-encoding section have been identified in diverse marine bacteria with shown iron-chelating activity (Koch et al., 2019b), indicating a similar functionality in Psym-73. The upstream spermidine-related section has ~40% amino acid similarity to the polyamine synthesis pathway of Vibrio, indicating a PecP-specific role in biofilm formation (Lee et al., 2009).

Ecological conclusions

The predominance of alginolytic pathways demonstrates alginate particles as preferred microbial substrate, whereas pectin was primarily a colonization scaffold. The establishment of similar communities contradicted our original hypothesis of community segregation by substrate preferences. On the contrary, the expression of alginate lyases when attached to pectin signify the concept of a ‘particlesscape’ encompassing cross-particle interactions. Such a scenario might resemble natural processes when algal polysaccharide exudates enter the water column, self-assemble into particles and sink to the seafloor. Under such circumstances, bacteria might utilize alginate even if attached to neighboring microhabitats, e.g. by secreting extracellular CAZymes or exploiting hydrolytic activity of co-occurring microbes. The predominance of few taxa indicates that polysaccharide availability stimulates only certain community members, outcompeting most other strains by their extensive CAZyme repertoire. Nonetheless, identification of a single MAG with pectin-specific dynamics suggests that numerically rare but competitive bacteria can establish in specific niches. Single-particle incubations and the application of ^13C-labelled substrates followed by NanoSIMS or stable isotope probing might answer these open questions in future studies. Altogether, our study illuminates central elements of the biological carbon pump in macroalgae-rich habitats, with implications for microscale ecology, niche specialization and bacteria–algae interactions.

Experimental procedures

Characteristics of polysaccharide particles

Custom polysaccharide particles consisting of alginate (CAS #9005-38-3) or pectin (CAS #9000-69-5), approximately 200 μm in diameter, were fabricated by geniaLab
Fig 7. MAG21 as candidate for pectin degradation.
A. Numbers of differentially abundant transcripts compared to the major PA-MAGs on PecP versus AlgP.
B. Elevated transcript abundances of glycoside hydrolase genes on PecP.
C, left panel: PUL with similarities to the pectinolytic operon BT4667–4673 in Bacteroides thetaiotaomicron. C, right panel: PUL encoding GH105 and GH73 genes plus a hypothetical protein with 60% amino acid identity to an alpha-amylase from Paraglaciecola arctica (UniProtKB accession K6ZD77; indicated by asterisk).
Seawater sampling and experimental set-up

Seawater was sampled from approx. 1 m depth above macroalgal forests at Helgoland Island (54.190556 N, 7.866667 E) in June 2017. Seawater was filtered through a 100 μm mesh, brought to the lab within 2 h, and filtered again through a 20 μm mesh to remove larger particles and organisms. Each 12 L of filtered seawater were distributed into 20 L Clearboy bottles (Nalgene, Rochester, NY) previously rinsed with the same seawater. Per bottle, 250 ml of the original seawater were added as additional nitrogen and phosphorous source to avoid limitation. Three experiments were set up in triplicate: (i) magnetic alginate particles and non-magnetic pectin particles, (ii) non-magnetic alginate particles and magnetic pectin particles, and (iii) control without particles (Supplementary Fig. 1). Each particle type was added at 3500 L⁻¹, resulting in ~42 000 particles per bottle. Bottles were incubated statically at 15°C (approx. in situ temperature) in the dark.

Sampling and nucleic acid extraction of particle-associated and free-living cells

250 ml of the original seawater were filtered onto 0.2 μm polycarbonate filters for determination of the ambient in situ community (start). Filters were flash-frozen in liquid nitrogen and stored at −80°C. Incubations were sampled after 24, 48 and 60 h. At each sampling point, bottles were mixed by inversion and ca. 550 ml withdrawn into 2 ml RNase-free microcentrifuge tubes. The supernatant was transferred to a separate tube and non-magnetic particles were removed by filtration through 5 μm polycarbonate filters. The flow-through was captured on 0.2 μm polycarbonate filters to obtain the FL community. All samples were directly flash-frozen in liquid nitrogen and stored at −80°C. Simultaneous extraction of DNA and RNA was done using a modification of Schneider et al. (2017). Purified DNA and RNA were sent on dry ice to DNASense (Aalborg, Denmark) for quality control and sequencing. For particles, several subsamples per replicate were pooled to obtain sufficient DNA and RNA (Supplementary Table 1).

16S rRNA gene amplicon sequencing

Briefly, the V3–V4 region of bacterial 16S rRNA genes was sequenced using primers 341F-806R (Sundberg et al., 2013) with MiSeq technology (Illumina, San Diego, CA). Internal company standards worked as expected (Supplementary Methods). Reads were classified into ASVs using DADA2 (Callahan et al., 2016) and taxonomically assigned using SILVA v132 (Quast et al., 2013). Rarefaction analysis showed that diversity was reasonably covered (Supplementary Fig. 9). Replicates were congruent per treatment and time, without significant differences in Bray–Curtis dissimilarities (PERMANOVA; p = 0.72 to 0.98). Furthermore, FL communities from AlgP and PecP were congruent as expected, and FL data combined in subsequent analyses. Alpha-diversity indices (richness, Shannon and inverse Simpson) were calculated using R package iNEXT (Hsieh et al., 2016).

Metagenomics

As amplicon data confirmed the consistency of replicates, DNA from the three AlgP, PecP and FL replicates at 24 and 60 h were pooled respectively. DNA was quantified using Qubit (Thermo Fisher Scientific) and fragmented to ~550 bp using M220 using miTUBE AFA fibre screw tubes (Covaris, Woburn, MA) for 45 s at 20°C with duty factor 20%, peak/displayed power 50 W, and cycles/burst 200. Libraries were prepared using the NEB Next Ultra II kit (New England Biotech, Ipswich, MA) and paired-end sequenced (2 × 150 bp) on a NextSeq system (Illumina). Adaptors were removed using cutadapt v1.10 (Martin, 2011) and reads assembled using SPAdes v3.7.1 (Bankevich et al., 2012). Genes were predicted using Prokka (Seemann, 2014) and assigned to KEGG categories using KAAS-SBH-GhostX (Moriya et al., 2007). CAZymes were predicted using dbCAN2
with CAZyDB v8 (Zhang et al., 2018), only considering hits with >80% coverage. Ammonium transporters were predicted by BLASTp of AmtB (P69681) in the Transporter Classification Database (Saier et al., 2016).

Metatranscriptomics

RNA was quantified in duplicate per sample using the Qubit BR RNA assay (Thermo Fisher Scientific). RNA quality and integrity were confirmed using TapeStation with RNA ScreenTape (Agilent, Santa Clara, CA). RNA was depleted using the Ribo-Zero Magnetic kit (Illumina) and residual DNA removed using the DNase MAX kit (Qiagen). rRNA removal was confirmed using TapeStation HS RNA ScreenTapes (Agilent). Sequencing libraries were prepared using the TruSeq Stranded Total RNA kit (Illumina), quantified using the Qubit HS DNA assay (Thermo Fisher Scientific) and size-estimated using TapeStation D1000 ScreenTapes (Agilent). For RNA from particle samples, four to five subsamples per replicate were pooled in equimolar concentrations and sequenced on a HiSeq2500 in a 1 × 50 bp Rapid Run (Illumina). As the first sequencing run did not deliver sufficient data for seven metatranscriptomes, a second run was performed on the same library. PCA confirmed consistent sequencing runs (data not shown), and read counts were subsequently aggregated. Raw fastq sequence reads were trimmed using USEARCH v10.0.2132 (Edgar, 2010) using -fastq_filter and settings -fastq_minlen 45 -fastq_truncqual 20. rRNA reads were removed using BBduk (http://jgi.doe.gov/data-and-tools/bb-tools) using the SILVA database as reference (Quast et al., 2013). Reads were mapped to the predicted genes using Minimap2, discarding reads with sequence identities <0.98. Relative transcript abundances were obtained by dividing raw counts by the length of each gene (RPK) and normalized by per-million scaling factors. Resulting transcripts per million (TPM) were summed per gene annotation (Supplementary Table 2). Differential transcript abundances were calculated on raw read counts using the default DESeq2 workflow in R v3.6 (Love et al., 2014; R Core Team, 2018) in RStudio (https://rstudio.com), only considering log2-fold changes >2 with \( p_{\text{adj}} < 0.001 \) (Supplementary Table 3).

MAG binning and analysis

MAGs were binned using MetaBat2 and mmgenome2 (Karst et al., 2016; Kang et al., 2019). Reads were mapped back to the assembly using Minimap2 v2.5 (Li, 2018), and the average coverage of each bin was calculated using mmgenome2 (weighted by scaffold sizes). Based on results from CheckM and GTDB-Tk (Parks et al., 2015; Chaumeil et al., 2020), we selected five near-complete MAGs (≥90% estimated genome completeness and ≤5% genome contamination) representing the major taxa in amplicon data (Table 1, Supplementary Table 4). Whole-genome comparison with type strains was carried out using the MiGA web application (Rodriguez-R et al., 2018). Normalized coverage in metagenomic data was calculated following Poghosyan et al. (2020) after multiplying the coverage of each MAG in every sample with a normalization factor (sequencing depth of the largest sample divided by the sequencing depth of each individual sample). A maximum-likelihood phylogeny based on 92 core genes identified using the UBCG pipeline (Na et al., 2018), including medium-quality MAGs (>70% estimated completeness/≤10% contamination) assigned to the same genus plus related genomes from public databases, was calculated using RaxMLHPC-Hybrid v8.2.12 with the GTRGAMMA substitution model and 1000 bootstrap replicates on the CIPRES Science Gateway v3.3 (Miller et al., 2010; Stamatakis, 2014). Genes were assigned to KEGG categories using KAAS, and pathways reconstructed from these predictions using KEGG Pathway Mapper (Moriya et al., 2007; Kanehisa and Sato, 2020). Gene annotations were refined using UniProtKB/Swiss-Prot (Boutet et al., 2016) by custom-BLAST in Geneious v7 (https://www.geneious.com). Genes for processing alginate and pectin monomers were predicted based on the fully reconstructed pathways in A. maculeodi and Gramella forsetii (Kabisch et al., 2014; Koch et al., 2019a). For comparative purposes, CAZymes of strains B3M02 and E3R01 (Enke et al., 2019) were re-annotated with dbCAN2 v8.0 and compared to MAGs Ten-26 and Psym-73 using custom-BLAST in Geneious v7 (https://www.geneious.com). Genes for processing alginate and pectin monomers were predicted based on the fully reconstructed pathways in A. maculeodi and Gramella forsetii (Kabisch et al., 2014; Koch et al., 2019a). For comparative purposes, CAZymes of strains B3M02 and E3R01 (Enke et al., 2019) were re-annotated with dbCAN2 v8.0 and compared to MAGs Ten-26 and Psym-73 using custom-BLAST in Geneious, only considering hits with >30% query coverage and >40% amino acid identity. ANIs between genomes were calculated using enveomics (Rodriguez-R and Konstantinidis, 2016). Prophages and biosynthetic gene clusters were predicted using PHASTER and antiSMASH 5.0 respectively (Arndt et al., 2016; Blin et al., 2019). Amino acid sequences of PL7 genes from Psym-73 were aligned using the MAFFT E-INS-i algorithm with default parameters (Katoh et al., 2002). A maximum-likelihood phylogeny with 500 bootstrap replicates was calculated using RaxML v8.0 (Stamatakis, 2014) and the WAG+G+F substitution model determined using ModelTest-NG (Darriba et al., 2020), both run on CIPRES (Miller et al., 2010).

Data availability

All sequencing data have been deposited at NCBI under BioProject PRJEB38771 (see Supplementary Table 1 for accession numbers of each sequence file).
metagenome contigs and near-complete MAGs, genes and translations, metatranscriptomic counts and the DNA–RNA extraction protocol are available at https://doi.org/10.5281/zenodo.4171148. R scripts and additional input files for reproducing the analysis are available at https://github.com/matthiaswietz/sweet-spheres. Major R packages used for analysis and visualization included phyloseq, ampvis2, tidyverse, ComplexHeatmap, gtools and PNWCColors (McMurdie and Holmes, 2013; Gu et al., 2016; Andersen et al., 2018; Wickham et al., 2019; Lawlor, 2020; Warnes et al., 2020).

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Supplementary Fig. 1.** A. Sampling location (cross) near Helgoland Island above dense macroalgal forests; schematically depicted in green. B. Conceptual overview of experimental setup using custom polysaccharide particles (insert). Magnetic particles were separated from their non-magnetic counterparts by magnetic selection (Supplementary Video 1) and frozen in cryotubes. FL communities were sampled by size-fractionated filtration of the supernatant (5 µm followed by 0.2 µm). Ambient seawater (start) and control samples (CTR) were directly filtered on 0.2 µm. Amplicon (0, 24, 48 and 60 h), metagenomic (24 and 60 h), and metatranscriptomic sequence data (60 h) were generated after different intervals of incubation.

**Supplementary Fig. 2.** Relative abundances of bacterial genera (top) and inverse Simpson alpha-diversity index (bottom) on alginate (AlgP) and pectin particles (PeCP), in the free-living fraction (FL), control incubations (CTR) and the ambient in situ community (start). Only genera with abundances >4% are shown.

**Supplementary Fig. 3.** Relative abundances of *Colwellia* and *Glaciecola* ASVs in PA and FL communities. Each colour corresponds to a distinct ASV.

**Supplementary Fig. 4.** Maximum-likelihood phylogeny of MAGs based on 92 single-copy core genes, including the five near-complete MAGs (>90% completeness, <10% contamination), medium-quality MAGs (>70% completeness, <10% contamination) assigned to the same genus, and other related genomes.

**Supplementary Fig. 5.** Degradation of alginate and pectin based on Hobbs *et al.*, 2019 and Koch *et al.*, 2019a. TBDR: TonB-dependent receptor; MFS: major facilitator superfamily transporter; TRAP: tripartite ATP-independent transporter; DehR: Deh reductase; KdgK: KDGP kinase; KdgA: KDGP aldolase; KdgF: responsible for uronate linearisation; KduL: 4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase; KduO: 2-deoxy-D-glucuronate 3-dehydrogenase; UxaA: alronate dehydratase; UxB: fructuronate reductase; UxC: glucuronate isomerase; DEH: 4-deoxy-L-erythro-5-hexoseulose uronate; KDG: 2-keto-3-deoxy-D-gluconate; KDGP 2-keto-3-deoxy-6-phosphogluconate.

**Supplementary Fig. 6.** Detailed architecture of PUL in MAGs.

**Supplementary Fig. 7.** A: Truncated alginolytic operon in *Glaciecola* MAG Gla-32 compared to the functionally characterized PUL in *Alteromonas macroleiodi* 83–1. BLASTp confirmed that missing genes were not encoded on other Gla-32 contigs. B: Hybrid biosynthetic gene cluster in *Psychromonas* MAG Psym-73 encoding a siderophore homologous to a functional cluster in *Alteromonas* sp. 76–1 (left section) as well as spermidine-related genes homologous to VC1623 and VC1624 in *Vibrio cholerae* (right section). Locus tags are shown inside the first and last gene.

**Supplementary Fig. 8.** Maximum-likelihood phylogeny of PL7 genes from *Psychromonas* MAG Psym-73, using BAB03312.1 from *Sphingomonas* as outgroup. Only some homologues showed substantial transcript abundances (right insert).

**Supplementary Fig. 9.** Rarefaction and coverage analysis of amplicon reads.

**Supplementary Table 1.** Metadata, sampling strategy, and statistics from amplicon, metagenome and metatranscriptome sequencing.

**Supplementary Table 2.** Complete overview of metagenomic genes, their abundance in metatranscriptomes (transcripts per million), and their assignment to CAZyme and KEGG categories.

**Supplementary Table 3.** Complete overview of differential transcript abundance analysis.

**Supplementary Table 4.** Complete overview of metagenome-assembled genomes.

**Supplementary Table 5.** Comparison of *Tenacibaculum*-MAG Ten-26 and *Psychromonas*-MAG Psym-73 with *Psychromonas* sp. B3M02 and *Tenacibaculum* sp. E3R01 from Enke *et al.* 2019 (https://doi.org/10.1016/j.cub.2019.03.047).

**Supplementary Table 6.** Composition of alginate and pectin particles.

**Supplementary Video 1.** Magnetic polysaccharide particles.