Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes

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Adaptive radiations are important drivers of niche filling, since they rapidly adapt a single clade of organisms to ecological opportunities. Although thought to be common for animals and plants, adaptive radiations have remained difficult to document for microbes in the wild. Here we describe a recent adaptive radiation leading to fine-scale ecophysiological differentiation in the degradation of an algal glycan in a clade of closely related marine bacteria. Horizontal gene transfer is the primary driver in the diversification of the pathway leading to several ecophysiologically differentiated Vibrionaceae populations adapted to different physical forms of alginate. Pathway architecture is predictive of function and ecology, underscoring that horizontal gene transfer without extensive regulatory changes can rapidly assemble fully functional pathways in microbes.
Adaptive radiations are thought to have played an important role in the diversification of life. They manifest as rapid ecological differentiation of a single clade of organisms in response to ecological opportunity thought to arise primarily from resource availability following extinctions or colonization of new habitats. A prime example are Darwin’s finches, which quickly diverged from a single ancestor into several, locally adapted species on the Galapagos Islands due to evolvability of beak shape, which allowed rapid adaptation to novel resources. In recent years, laboratory evolution and mesocosm studies using microbes have provided experimental support for ecological opportunity triggering adaptive radiations. However, for microbes in the wild, adaptive radiations, both ancient and recent, have remained difficult to document. First, the details of ancient diversifications are nearly impossible to reconstruct, since past ecological opportunities are often unknowable, and horizontal gene transfer (HGT) can erode phylogenetic signal. Furthermore, it is even questionable whether adaptive radiations might be possible in contemporary environments, considering that the long co-evolutionary history of microbes and their resources has led to high niche filling. As a consequence, we do not know genetic mechanisms and ecological opportunities that could give rise to adaptive radiations in complex natural environments.

Here we ask whether a group of very closely related but ecologically differentially associated bacterial populations show the characteristics of an adaptive radiation, including rapid diversification of a single clade into multiple, ecologically differentiated clades, associated with traits adaptive towards environmental opportunities. These populations were originally identified as genotypic clusters in protein-coding marker genes with differential distribution among size fractions within the same water samples indicating association with different resource types such as dissolved or particulate organic matter and zoo- or phytoplankton. Subsequent work has established that these clusters also act as gene flow, and behavioural units suggesting that they possess many attributes commonly associated with sexual species. However, because our sampling scheme considers only bacteria co-existing in small-scale microhabitats, we designate them as populations to which we assign species names if a previously described type strain falls within the genotypic cluster identified as a distinct population.

Our test case is a clade of very closely related Vibrionaceae isolates, which we previously hypothesized to comprise at least seven recently speciated populations based on their genetic and environmental structure. We show that this clade rapidly diversified into population-specific ecophysiologically types specialized for the degradation of different physical manifestations of alginate (chain length, solubility and concentration) of the same algal glycan. This specialization is manifest as unique pathway configurations that arose by extensive horizontal gene transfer and are highly predictive of metabolic performance. We first reconstruct the evolution of the different pathway types and characterize their physiological properties. We then show that environmental associations are consistent with the physiological predictions and propose a model of glycan degradation involving the evolution of interacting populations.

Results

Alginate degradation pathways differentiate populations. We first asked whether adaptive changes can be hypothesized based on comparison of 84 genomes representing the Vibrionaceae populations, including a clade of seven very recently diverged populations (crown group) (Fig. 1, grey box). This analysis highlighted a pathway specific for the degradation of the brown algal glycan alginate as having undergone extensive evolutionary changes across the majority of populations (Fig. 1a,b; Supplementary Fig. 1). These include both population-specific presence and absence of the pathway, as well as major differences in its architecture. For example, several populations contain a canonical pathway consisting of four polysaccharide lyase (PL) families, while others lack up to three of the four lyase families (Fig. 1b). These four families perform different molecular functions in the alginate pathway: alginate lyases (Aly) PL6 and PL7 initiate the extracellular lysis of the polymer, and members of two oligoalginolyase (Oal) families PL15 and PL17 complete degradation into monomers. The latter two gene families are the keystone carbohydrate active enzymes of the core pathway, since they generate sugar monomers that can be further catabolized, and their absence abolishes pathway functionality. These initial observations suggested the possibility of a fine-grained analysis of the evolutionary history and potential adaptive significance of the alginate pathway differentiation.

Pathways have assembled primarily horizontally. Cursory inspection of the alginate pathway across our Vibrionaceae populations appears consistent with an ancient, single HGT since a core set of alginate degradation genes is present in a majority of the clade including the deeply branching Aliivibrio (Fig. 1a; Supplementary Figs 1 and 2). However, detailed phylogenetic reconstruction (Methods) that includes an additional 395 high-quality genomes obtained from Genbank for reference (Supplementary Fig. 3) reveals an unexpectedly complex history (Fig. 1d,e). In most cases, multiple copies of each alginate lyase family represent independently evolving subfamilies that did not arise by duplication within the Vibrionaceae (see Methods for statistical support for definition of subfamilies). In fact, there is little vertical descent and the majority of clades with alginate degradation pathways acquired both Oal and Aly genes horizontally (Fig. 1d,e). Across all our populations, transfer of Oal genes was so common that every population exchanged at least one gene copy with at least one other population (Fig. 1d). Even among the seven closely related populations of the crown group we estimate three independent initial acquisitions of Oal subfamilies from a variety of sources followed by lateral spread among populations and acquisitions of new subfamilies (Fig. 1a,d). Moreover, Alys and Oals are distributed across multiple regions on chromosome 1, chromosome 2 and a putative extrachromosomal element in one Vibrio breoganii (FF50) and one Vibrio sp. F13 (9CS106) isolate with nearly closed genomes. These regions are significantly enriched in genes annotated as mobile elements, transposases and integrases (hypergeometric test, \( P = 0.0019 \)). Some of these regions also display significantly decreased GC content consistent with the recent introduction of foreign DNA (Supplementary Table 1). Hence multiple lines of evidence reject the seemingly ancient acquisition and subsequent vertical modification of the core pathway and instead suggest multiple recent acquisitions and transfers.

The core pathway of Oal genes was extended in a surprisingly rapid and complex sequence of events by independent acquisitions and transfers of Aly families PL6 and PL7. Similar to the Oals, Aly genes also spread extensively within the crown group by independent acquisition and transfer. However, these genes were lost in a lineage-specific manner within Vibrio tasmaniensis and Vibrio lentus (Fig. 1d,e). Furthermore, Vibrio sp. F10 never acquired Aly genes despite possessing both Oals (Fig. 1d,e). The more basal Vibrio groups, V. breoganii, V. rumoiensis and the Aliivibrio, all independently acquired these genes as well and transferred a number to the crown group (Fig. 1e). Taken together, several different pathways were assembled by an
evolutionary Ping Pong of rapid back and forth transfers among lineages; the pace of this is evident in the crown group of vibrios that are nearly indistinguishable in ribosomal protein gene sequences yet contain populations that have lost the pathway or acquired a range of Alys in addition to the core set of Oal genes (Fig. 1; Supplementary Fig. 1).

The considerable variation in Aly gene copy number, which arose primarily by acquisition and loss rather than duplication,
differentiates pathways functionally, highlighting the key role of HGT and the flexible genome in niche differentiation among populations. In particular, while PL6 and PL7 gene copies are absent in several lineages, they are especially abundant in *V. breganii, Vibrio cyclitrophicus, Vibrio splendidus* and *Vibrio* sp. F13. In *V. breganii*, the pathway underwent the most significant expansion, involving duplications of PL6 and PL7 genes in addition to several PL7 gene transfers from multiple sources (Fig. 1d,e). Within each population, further variation in PL7 copy number also exists (Supplementary Figs 1 and 4) but it is unclear whether such variation is due primarily to transfer, loss or duplication. Regardless, these observed differences in gene copy number have important physiological consequences; populations possessing more Aly gene copies showed increased enzyme expression (Supplementary Fig. 5) and enzymatic activity when exposed to alginate (Fig. 2). Because many of the genes are distributed across different regions in the genome and hence not co-regulated, increased activity rapidly evolved, in large part by gene acquisition and expression. The gene acquisition we observed is reminiscent of molecular cloning and exemplifies gene acquisition and expression. The cells were lysed to determine the total, cell enzyme expression. The cells were lysed to determine the total, cell associated alginate lyase activity by measuring the increase of absorption at 235 nm with alginate as enzyme substrate. The alginate lyase activity of each strain was normalized against the optical density of the respective cell culture measured at 600 nm. The experiment was carried out in triplicate and the error bars display the standard deviation of the mean.

**Populations possess different ecophysiological strategies.** We next asked how differences in pathway architecture might shape ecological niches at the population level. We first hypothesized that populations possessing only Oal genes may only have limited ability to utilize oligoalginate molecules. We therefore performed growth experiments on alginate of high (degree of polymerization, Dp > 50), medium (Dp ~ 20), and low (Dp ~ 3–4) molecular weight, which reflects the potential resource space, since extracellularly released oligosaccharides are a byproduct of degradation. Consequently, cells can access these products only if they are able to grow on alginate. A subset of isolates displayed long lag phases – over 24 h in some cases – that increased with polymer length (Fig. 4b–d, for example, 1F157 and ZF211). We hypothesized that short lag phases are enabled by broadcasting Aly into the three-dimensional polymer matrix while long lag phases occur in isolates that tether the enzymes to the cell allowing only access to the two-dimensional polymer surface. Consistent with this hypothesis, broadcast alginate lyase activity was high among isolates with short lag phases (Fig. 4b–d, for example, 13B01 and 12B01), but comparatively low in isolates with longer lag phases (Fig. 4b–d, for example, 1F157 and ZF211). Notably, membrane-bound and intracellular alginate lyase activity was comparable among isolates, regardless of lag phase length (Fig. 4b,c). Furthermore, in a plate-based assay intended to visualize broadcasted alginate lyase activity, isolates with short lag phases created large halos of lyase activity that extended far beyond the colony boundary (Fig. 4d). These halos were small or absent for isolates displaying long lag phase. Interestingly, this long lag phase phenotype arose independently as a population-level characteristic among all *Alivibrio fischeri* isolates capable of degrading alginate and a within-population polymorphism in *V. splendidus* displayed by ~5% of isolates. Our results also provide an explanation for recent observation of lag phases among some *Alteromonas macleodii* isolates and suggest this phenotype might be common.

Our plate-based enzymatic broadcasting assay also revealed additional polymorphisms in the strength of the broadcasting phenotype. Some isolates appeared to be ‘super broadcasters’ with unusually large halos, indicating their superior ability to degrade high-molecular weight alginate (Fig. 4d). This phenotype can be traced back to the acquisition of a PL7 in a subset of *V. splendidus*, including strain 13B01. Interestingly, a super broadcaster phenotype was recently bioengineered for production of bioethanol from algal biomass by combining the alginate pathway of *V. splendidus* 12B01, a low broadcaster, with an engineered PL7 enzyme that is secreted by *Escherichia coli*. Hence our analysis demonstrates that nature found an identical solution to the problem of rapid access of the insoluble polymer and highlights fine-scale physiological differences as a resource for bioengineering. Ecologically, isolates that broadcast enzymes may act as ‘pioneers’, which have a competitive advantage when colonizing native substrates. By contrast, while isolates with long lag phases possess the full repertoire of alginate lyases (both Alys and Oals), they likely grow more rapidly in the presence of isolates that broadcast alginate lyases into the environment. We therefore refer to them as ‘harvesters’, since they may harvest the fruits of enzymes sown by pioneers.

The observed physiological variation suggests differential adaptation of the populations to alginate and its partial
degradation products, which ultimately derive from algal cell walls. Yet Vibrionaceae are generally regarded to be animal (especially zooplankton) associated, including many facultative pathogens. Hence, we tested to what extent possession of the alginate pathway is linked to association with dead algal biomass. To identify habitat association, we collected small particles recognizable under the microscope as algal detritus, and, for comparison, live and dead zooplankton (primarily copepods) during the fall and spring season, and obtained isolates on selective media (Fig. 1c). Presence of Aly genes is strongly associated with presence on algal particles (Fisher’s exact test, $P = 1.68 \times 10^{-7}$). Furthermore, at least six populations (A. fischeri, V. breoganii, Vibrio sp. F13, V. tasmaniensis, V. lentus and V. splendidus) overlap in their habitat preferences by occurring on algal detritus, albeit V. splendidus was more strongly represented in spring, that is, cold water samples. Importantly, none of the populations that lack the entire alginate degradation pathway could be isolated from algal detritus. Moreover, several populations capable of alginate degradation, which were absent from algal biomass, might occupy different environmental microhabitats. For example, V. cyclitrophicus has previously been hypothesized to co-occur with unicellular algae in

![Figure 3](image-url)
Figure 4 | Membrane-bound versus broadcasted alginate lyases dictate growth lag time. (a) Growth curves of isolates representing distinct pathway architectures on high- (degree of polymerization, Dp > 50), medium- (Dp ~ 20), or low- (Dp ~ 3–4) molecular weight alginate. Low and medium molecular weight alginate was further purified into mannuronate (M)- or guluronate (G)- enriched fractions. (b) Quantified lag time differences between strains. (c) The cellular milieu was fractionated into extracellular (secreted), membrane-bound, and intracellular components. For each fraction, alginate lyase activity was measured using a bulk enzymatic activity assay (Methods). Among isolates assayed, those with longer lag phases displayed reduced broadcasted alginate lyase activity, despite similar levels of intracellular- and membrane-bound alginate lyase activity. Bar diagrams represent averaged technical replicates (n = 3). Error bars represent standard deviations of the mean. One unit of activity defines an increase of 1.0 in absorbance at 235 nm per min. (d) Broadcasted alginate lyase activity measured independently with a plate-based assay (Methods). The size of the halo indicates the degree of broadcasted alginate lyase activity after a fixed period of time. Bar diagrams represent means of technical replicates (n = 5) and error bars represent s.d.

Discussion
Combining phylogenetic, physiological, and environmental data, we suggest that a horizontally acquired alginate degradation pathway has undergone an adaptive radiation, which may mitigate competitive exclusion and enable a degradation cascade involving three ecophysiological types. First, ‘pioneers’ – alginate degraders with Oals and broadcasted Alyss – colonize and degrade the intact polymer, thereby creating more soluble forms of the polymer and oligomers (Fig. 5). In the process, pioneers construct a niche for two other types of populations, which we refer to as ‘scavengers’ and ‘harvesters’. Scavengers, which only have Oals, are ‘cheaters’ that cannot degrade alginate directly, but can take advantage of small oligomers (Dp ~ 3–4) produced extracellularly by the pioneers. By contrast, harvesters represent an intermediate between the pioneers and scavengers. Like pioneers, harvesters possess both Oals and Alyss. However, instead of broadcasting the Aly enzymes, harvesters tether Alyss to their cell surface. Furthermore, like scavengers, harvesters may also take advantage of small alginate oligomers produced by pioneers.

In theory, there are many mechanisms that might support the coexistence of these three ecological strategies in nature. For pioneers and scavengers, these include spatial structure25,26 and asymmetric access to nutrients27. For harvesters, their lack of broadcast enzymatic products leads to a growth detriment (through long lag phases during growth on high-molecular weight alginate), but also makes them less likely to share their enzymatic degradation products. Thus, harvester populations may not be as prone to invasion by scavengers – as has been recently described for select human gut Bacteroidetes28 – thereby allowing them to coexist with pioneers and scavengers. Finally, even different pioneer populations (V. breogami and Vibrio sp. F13) are further ecologically differentiated by enzymatic activity levels, stemming from distinct pathway architectures (Fig. 2; Supplementary Fig. 5), which may allow for their coexistence. Nonetheless, interactions may lead to fluctuations in populations and further work will be required to determine how stably pioneers, scavengers, and harvesters can coexist in the wild.

Our analysis shows that a very general ecological opportunity creates a surprisingly strong selective regime as evidenced by the
rapid, repeated evolution of different ecophysiological types among closely related bacteria. This finding underscores the general evolvability of microbes consistent with adaptive radiation documented in experimental evolution studies. The mechanism, however, appears fundamentally different. HGT assemblies highly nuanced functional pathways from different sources with apparent speed and facility. This includes increase in enzymatic activity, which appears driven by acquisition of gene copies, rather than changes in regulation. This importance of mechanism, however, appears fundamentally different. HGT is consistent with adaptive radiation of microbes consistent with diversifying populations, suggesting that such variation must be explored in detail if we are to understand the rules of microbial community assembly.

Methodology

Isolation and culture conditions. Strains tested here originated from previous studies on the ecological population structure of Vibrionaceae. Briefly, isolates were obtained either from size fractionated water samples, handpicked algal detritus or plating samples on Vibrio-selective marine TCBS media. Individual colonies were picked and purified by re-streaking three times.

Genome sequencing and assembly. DNA from strains 1S159, 1S128, 1S175, 1S165, 5F7, FF27, ZF47, SF23, SF33, SF97, ZF73, SF36, ZF25 and SF146 was extracted with the DNeasy Blood and Tissue Kit using the protocol for Gram-negative bacteria. Initial assemblies were performed using the SMRT Cell DNA Library Preparation Kit (Ilumina). Sequence reads were demultiplexed using a custom python script and imported into CLC Genomics Workbench 8.0 (http://www.clcbio.com) for further processing and assembly. Adaptors and low-quality regions were trimmed from the demultiplexed sequences and overlapping paired reads were merged. The final assemblies were performed using the CLC assembler with read mapping correction. Draft genome assemblies were available for 63 strains indicated in Supplementary Table 2. Assemblies for some of these strains were refined by resequencing. DNA was isolated and short read sequencing libraries were performed by random shearing per Illumina’s protocol and sequenced short (51–71 bp) single-end reads on an Illumina GAII, or paired-end reads on an Illumina GAII (ref. 11). Genome assemblies for strains 12B01 and 12G01 were corrected with these short read sequences. Additionally, long-insert libraries were prepared for 11 genomes representing different populations (1F35, ZF14, ZF29, 9Z13, 12B09, SS149, SS101, FF33, ZF211, ZS17 and FF500) to aid in scaffolding assemblies. Final assemblies using these reference strains was performed by mapping short read sequences to the most closely related long-insert assembly as previously described. This hybrid short read and long-insert scaffolding approach was also used to generate de novo assemblies for six additional strains (SF59, ZF57, FF273, IA66, 9CS112 and 13B01).

Strains 9CS106 and FF50 were sequenced using the PacBio RSII at the Yale Center for Genome Analysis. Initial assemblies were performed using the SMRT Portal Software at the MIT BioMicro Center and the HGAP Assembly #2 algorithm. When appropriate, assemblies were circularized with the minimus software package and assemblies were refined using the Resequencing #1 algorithm in SMRT Portal. This assembly was corrected by mapping the short read Illumina sequences in CLC Genomics Workbench 8. This corrected assembly resulted in two closed chromosomes and one circular extrachromosomal element in FF50, and two nearly closed chromosomes and two circular extrachromosomal elements in 9CS106.

An additional 395 Vibrionaceae and 51 Shewanella genomes were retrieved from Genbank (ftp://ftp.ncbi.nlm.nih.gov/genomes/) for a total of 530 genomes analysed.

Reference phylogeny construction. Ribosomal proteins were identified using hidden Markov models (HMMs) constructed from a previously published alignment of bacterial and archaeal ribosomal proteins. These were searched against all ORFs from the 84 sequenced genomes with hmmsearch (http://hmmer.org). ORFs matching ribosomal proteins with an e-value greater than 10–16 were excluded from further analysis. Paralogous ribosomal proteins were also excluded. All remaining ribosomal proteins present in at least 50% of all isolates were aligned using the MAFFT-L-INS-i algorithm with default parameters. Corresponding nucleotide sequences were aligned with the protein alignment as a guide using MAFFT. A maximum-likelihood tree was constructed under the GTR+G+F model of sequence evolution in RaXML. The tree was rooted using Shewanella as an outgroup. A relative timed tree was created from the maximum-likelihood tree using RELTIME.

Identification of alginate lyase domains. HMMs of Aly and Oal as Vibrionaceae-specific clades that are divided by non-Vibrionaceae outgroups, however, so closely related that two alternative scenarios were possible: either horizontal acquisition of two different (albeit closely related) subfamilies or vertical evolution within the Vibrionaceae and transfer to the outgroups. We therefore tested the robustness of these two subfamilies to arrive at the most conservative scenario using the following methods: (1) determining bootstrap support values for Vibrionaceae clades and their non-Vibrionaceae outgroups; (2) testing the inferred ML topology against a more conservative topology (that is, a topology that grouped Vibrionaceae genes into a single clade) with the approximately unbiased test (AU test); (3) comparing branch lengths within a subfamily phylogeny to branch lengths within a related subfamily with an origin supported by criteria 1 and 2. We performed these analyses on the PL17, PL6 and PL7 phylogenies. PL15 was not included in this analysis as only one PL15 subfamily was found. For PL17, originally five subfamilies were inferred. However, bootstrap support for these subfamilies was always low (< 60). We tested the alternative hypothesis that subfamilies 1 and 3 were a monophyletic group with the AU test. This topology was not significantly different from the inferred ML topology (P = 0.339). Performing the same test on subfamilies 4 and 5 yielded the same result (P = 0.118). However, the alternative topology with subfamilies 1, 2, 3, 4 and 5 as a monophyletic clade was significantly different from the ML topology (P = 7 × 10–60) and we were able to reject that alternative hypothesis. Therefore we concluded that there are two independently evolving subfamilies of PL17. For PL6, originally two subfamilies were inferred. Subfamily 1 was well-supported by bootstraps (97) while subfamily 2 was not (42). We tested the alternative hypothesis that subfamilies 1 and 2 were a monophyletic group and found that this topology was significantly different from the inferred ML topology (P = 2.3 × 10–5). Therefore we concluded that there are two independently evolving subfamilies of PL6.

For PL7, originally 14 subfamilies were inferred. Subfamilies 1 and 14 were significantly supported by high bootstraps (90% and 90, respectively). We tested the alternative hypothesis that subfamilies 1 and 14 form a large clade along with a number of non-Vibrio members. Because of this, testing an alternative hypothesis was not appropriate given the number of possible alternatives available. We took advantage of the fact that subfamily 14 originated in the ancestor of Aliivibrio and Vibrio, making it the oldest of all Vibrionaceae PL7s tested. The longest root-to-leaf branch length in this subfamily is indicative of the amount of time that should be excluded in any Vibrionaceae-specific PL7 subfamily. Using this value (0.66 substitutions per site) as a cutoff, we were able to recover subfamilies 3–8 with UPGMA clustering by phylogenetic distance (Supplementary Fig. 10). However, subfamilies 9–12 were merged into a single subfamily. The distance between subfamily 2 and all other subfamilies greatly exceeded the cutoff described above. Therefore we concluded that there are 11 independently evolving subfamilies of PL7.

The final tree with presence/absence information of lyases and each of the lyase gene trees were visualized using the Interactive Tree Of Life.

Phylogenetic reconciliation. The Aly and Oal gene trees were reconciled against the reference phylogeny using AnGST with the following cost parameters: loss = 1.0, duplication = 2.0, HGT = 3.0 (ref. 40). The optimal reconciliation was the reconciliation with the lowest total event score. We also modified the AnGST model of sequence evolution in RaxML. The tree was rooted using Shewanella as an outgroup. A relative timed tree was created from the maximum-likelihood tree using RELTIME.

Statistical support of alginate lyase subfamilies. We initially defined subfamilies of each Aly and Oal as Vibrionaceae-specific clades that are divided by non-Vibrionaceae outgroups, however, so closely related that two alternative scenarios were possible: either horizontal acquisition of two different (albeit closely related) subfamilies or vertical evolution within the Vibrionaceae and transfer to the outgroups. We therefore tested the robustness of these subfamilies to arrive at the most conservative scenario using the following methods: (1) determining bootstrap support values for Vibrionaceae clades and their non-Vibrionaceae outgroups; (2) testing the inferred ML topology against a more conservative topology (that is, a topology that grouped Vibrionaceae genes into a single clade) with the approximately unbiased test (AU test); (3) comparing branch lengths within a subfamily phylogeny to branch lengths within a related subfamily with an origin supported by criteria 1 and 2. We performed these analyses on the PL17, PL6 and PL7 phylogenies. PL15 was not included in this analysis as only one PL15 subfamily was found. For PL17, originally five subfamilies were inferred. However, bootstrap support for these subfamilies was always low (< 60). We tested the alternative hypothesis that subfamilies 1 and 3 were a monophyletic group with the AU test. This topology was not significantly different from the inferred ML topology (P = 0.339). Performing the same test on subfamilies 4 and 5 yielded the same result (P = 0.118). However, the alternative topology with subfamilies 1, 2, 3, 4 and 5 as a monophyletic clade was significantly different from the ML topology (P = 7 × 10–60) and we were able to reject that alternative hypothesis. Therefore we concluded that there are two independently evolving subfamilies of PL17. For PL6, originally two subfamilies were inferred. Subfamily 1 was well-supported by bootstraps (97) while subfamily 2 was not (42). We tested the alternative hypothesis that subfamilies 1 and 2 were a monophyletic group and found that this topology was significantly different from the inferred ML topology (P = 2.3 × 10–5). Therefore we concluded that there are two independently evolving subfamilies of PL6. For PL7, originally 14 subfamilies were inferred. Subfamilies 1 and 14 were significantly supported by high bootstraps (90% and 90, respectively). We tested the alternative hypothesis that subfamilies 1 and 14 form a large clade along with a number of non-Vibrio members. Because of this, testing an alternative hypothesis was not appropriate given the number of possible alternatives available. We took advantage of the fact that subfamily 14 originated in the ancestor of Aliivibrio and Vibrio, making it the oldest of all Vibrionaceae PL7s tested. The longest root-to-leaf branch length in this subfamily is indicative of the amount of time that should be excluded in any Vibrionaceae-specific PL7 subfamily. Using this value (0.66 substitutions per site) as a cutoff, we were able to recover subfamilies 3–8 with UPGMA clustering by phylogenetic distance (Supplementary Fig. 10). However, subfamilies 9–12 were merged into a single subfamily. The distance between subfamily 2 and all other subfamilies greatly exceeded the cutoff described above. Therefore we concluded that there are 11 independently evolving subfamilies of PL7.

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unaffected by different birth scenarios. For this subfamily we chose to depict the
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Mobile element analysis. All genes within the 9CS106 and F500 genomes were
annotated using the srr_assign_using_falgams script from RAST. Regions containing Aly or Oal genes within were defined as any stretch of DNA where an Aly or Oal was no farther than 5,000 bp from the next Aly or Oal. A hypergeo-
metric test as implemented in Python was used to compare the occurrence of genes
comparing each region’s GC content to the GC content of the chromosome or
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Aly or Oal was no farther than 5,000 bp from the next Aly or Oal. A hypergeo-
metric test as implemented in Python was used to compare the occurrence of genes
imaged and subsequently removed by scraping. To image the secreted enzyme
activity, the plates were rinsed two times for ten minutes with MilliQ water at 20°C
to remove residual cells. After this washing step the plates were incubated with
50 ml of 10% cetylpyridinium chloride (Sigma) solution for 20 min, while gently
shaking at 20°C. The plates were washed twice with MilliQ water for 20 min to
remove unbound cetylpyridinium chloride and to increase contrast. Secreted
enzyme activity became visible by a dark halo on an opaque background. The area
of the colony and the area of the corresponding alginase activity halo was
measured with ImageJ.
RT-qPCR of Vibrio alginase lyase and oligoalginate lyase genes. Selected Vibrio
isolates were grown in triplicate with glucose as the sole carbon source until the
cells reached mid-log phase. Subsamples were taken from the cultures and
immediately preserved using RNAProtect (Qiagen) to determine the baseline
eXpression. The absence of alginate was confirmed. Cultures were then spun down, washed, and
re-dissolved into minimal media with alginate oligoalgicates as the only carbon
source. The cultures were allowed to grow for another two hours before samples
were again taken and preserved. Total RNA of all samples was isolated using the
RNeasy kit (Qiagen) and treated using the TURBO DNA-free Kit (Life Technol-
ologies) to remove all contaminating DNA. RNA purity was confirmed by showing
DNA was not detectable in any sample after 35–40 rounds of PCR. First-strand
cDNA synthesis was carried out using the SuperScript III Reverse Transcriptase kit
(Invitrogen). All reverse transcription reactions were carried out using 100–150 ng
total DNA per reaction and random hexamer primers. Transcript measurement
Aly or Oal genes were performed on a CFX96 detection system (Bio-Rad) using SYBR
select master mix (Life Technologies) and primers listed in Supplementary Table 3. Gene expression analysis was performed using
REST-2009 software (Qiagen) with Rp0D and GyrB as reference genes.
Size fractionation of alginate. To prepare alginate of different molecular weight
for growth experiments, low viscosity sodium alginate (Sigma #A2158) was size
fractionated with chemical and enzymatic approaches. For the production of
homopolymetric blocks of alginate with a Dp of ∼Dp20, the chemical method
adapted from Han et al. was used. Briefly, the alginate was heated to 100°C in
0.3 M HCl for 20 min. After 20 min, the collected alginate by gravity
filtration through cotton cloth. The soluble material (containing the
alginate was fractionated by addition of diluted HCl to the alginate solution until
the pH reached 2.85, inducing separation into a precipitate and supernatant
fractions enriched in mannuronate and guluronate, respectively. Both fractions
were neutralized with NaOH to obtain a pH of 7 followed by dialysis against MilliQ
water with dialysis tubing (MWCO, 1 kDa). Following dialysis, the guluronate- and
mannuronate-enriched alginate fractions were precipitated with ethanol, dried at
60°C, and re-dissolved in MilliQ water. The solutions were frozen at ∼80°C and
lyophilized to obtain fine powder. Unless otherwise indicated, all steps were carried
out at 20°C.
To prepare oligosaccharides with a Dp of Dp ∼3–4 the mannuronate and
guluronate enriched fractions were dissolved to obtain a concentration of 0.5%
(w/v) in 100 ml of 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS)
buffer (pH 7.5) and 0.5% sodium azide. This solution was amended with alginic acid
(Aly) from Flavobacterium sp. [4.2.2.3] (Sigma, A1603) at a concentration of
0.1 M. The alginic acid was fractionated by addition of diluted HCl to the alginic acid solution until the
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enriched in guluronate and mannuronate, respectively. Both fractions
were neutralized with NaOH to obtain a pH of 7 followed by dialysis against MilliQ
water with dialysis tubing (MWCO, 1 kDa). Following dialysis, the guluronate- and
mannuronate-enriched alginate fractions were precipitated with ethanol, dried at
60°C, and re-dissolved in MilliQ water. The solutions were frozen at ∼80°C and
lyophilized to obtain fine powder. Unless otherwise indicated, all steps were carried
out at 20°C.
Growth assays. To determine growth rates on different types of alginic acid
substrates, Vibrio isolates were grown in triplicate with alginic acid polysaccharide,
fractionated mannuronate (Dp ∼20 or ∼5) or guluronate (Dp ∼20 or ∼5),
or glucose as a control. All substrates were dissolved at 0.1% (w/v) concentration in
growth media (Marine Broth 2216 (DIFCO) or Marine Broth 2216 (DIFCO) plus
0.25% of low viscosity sodium alginate (A2158, Sigma). After 24 h of incubation at 20°C, the colonies were
imaged and subsequently removed by scraping. To image the secreted enzyme
activity, the plates were rinsed two times for ten minutes with MilliQ water at 20°C
to remove residual cells. After this washing step the plates were incubated with
50 ml of 10% cetylpyridinium chloride (Sigma) solution for 20 min, while gently
shaking at 20°C. The plates were washed twice with MilliQ water for 20 min to
remove unbound cetylpyridinium chloride and to increase contrast. Secreted
enzyme activity became visible by a dark halo on an opaque background. The area
of the colony and the area of the corresponding alginase activity halo was
measured with ImageJ.
Enzyme localization experiments. To localize alginase lyase activity in different
cell compartments, we measured alginase lyase activity in intracellular, membrane-bound and extracellular proteomes (after Method I in Thein et al.44). Strains were
grown for 24 h at 20°C in Marine Broth 2216 (DIFCO) and cells pelleted from 1 ml
subsamples by centrifugation at 3,000g for 5 min. The pellet was washed with sterile
filtered artificial seawater (Sea Salts, Sigma) and washed cells were added to 100 ml of
10 mM MOPS buffer at 20°C with 0.1% (w/v) sodium azide and 0.5% sodium
azide, pH = 7.5) containing 0.1% alginic polysaccharide (Sigma #A2158). For
the activity curves, 10 µl of bacterial lysate was incubated with 200 µl of the activity
buffer at 27°C. The absorption of the solution at 235 nm was used to quantify
polysaccharide degradation by increased absorption of the new double bond in the
non-reducing end sugar. A measurement was taken every minute up to 30 min.
The alginase degrading ability of each strain was determined by dividing the slope
of the linear part of its activity curve by its OD 600 measurement.

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fold in a centrifugal concentrator (Vivaspin) with a 10-kDa cutoff. The cell pellets were suspended in 500 µl of a buffer containing 0.2 M Tris-HCl (pH 8), 1 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA) to which 100 μl of lysozyme (5 mg ml⁻¹ in MilliQ water, Sigma) was added. The cell suspension was vortexed and incubated for 5 min at 20 °C after which 2 ml of MilliQ water was added. The cells were lysed by adding 3 ml of a solution of 50 mM Tris-HCl (pH 8), 2% (w/v) Triton X-100, 10 mM MgCl₂ and 50 μl of DNAsel (Applichem, 1 mg ml⁻¹ in MilliQ water). The suspension was gently mixed and stored on ice until it cleared. The lysed cells were ultra-centrifuged at 40,000g for 30 min at 4 °C to pellet the outer membrane fraction. The supernatant was stored on ice. The pellet was washed in 750 µl of the lysis buffer with no added DNase. The pellet was ultra-centrifuged at 40,000g for 30 min at 4 °C. After centrifugation the pellet was washed three times with 500 µl MilliQ water before it was stored on ice until the activity measurements were carried out.

For activity assays the outer membranes were suspended in 250 µl of 20 mM Tris (pH 7.5), 0.1% Tween 20 and 5 mM dithiothreitol (DTT). The activity measurements were carried out in a buffer containing 50 mM Tris (pH 7.5), 2 mM calcium chloride, 0.1 mM alginate and 0.5 M sodium chloride. 20 μl of each sample (concentrated supernatant, cell lysate and the membrane fraction) was added to 180 µl of activity buffer. The increase in absorbance at 235 nm was measured for 30 min with an absorbance reading every minute in Costar UV transparent microtiter plates (Corning #3635) at 25 °C.

**Sampling of algal detritus and zooplankton.** Algal detritus and zooplankton were collected from Plum Island Sound Estuary, Ipswich, MA in the spring and fall of 2007 as previously described. Briefly, algal detritus particles and zooplankton were collected by filtering one hundred liters of seawater through a 64 µm mesh net. Eight replicate 1001 samples were collected in each season, two samples per day. Samples were rinsed three times with sterile seawater, washed into a 50 ml conical tube, and kept at ambient temperature in the dark until processing ~ 2 h later. Algal derived particles, as well as living and dead zooplankton, were picked from each 1001 concentrate. All collected algal particles and zooplankton were washed three times with sterile seawater, after which they were homogenized in a tissue grinder. Subsequently, these lysates were diluted in sterile seawater and filtered onto 0.2 µm filters (Pall).

**Bacterial isolation and gene sequencing.** Homogenates of algal particles and zooplankton were plated on TCBS media (BD Difco TCBS with 1% NaCl added) to isolate *Vibrio* strains, as previously described. After colonies were allowed to grow, they were re-streaked three times, alternating between 1% Tryptic Soy Broth (TSB) media (BD Bacto with 2% NaCl added) and marine TCBS media. For identification and assignment to previously identified populations, the 16S rRNA gene and three protease-coding genes (*mdh*, *mlp* and *mdf*) were sequenced as previously described.

**Data availability.** Bacterial genomes that support the findings of this study have been deposited in GenBank with the accession numbers presented in Supplementary Table 2. *Vibrio* *hsp60*, *adk*, and *mdf* sequences have been deposited in GenBank under accession nos. GQ988782 to GQ990534 (*hsp60*), GQ990535 to GQ990567 (*adk*), GQ992298 to GQ994040 (*mdf*). All other relevant data supporting the findings of this study are available from the corresponding author upon request.

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J.-H.H., M.S.D., A.H., C.H.C., M.F.P. and E.J.A. designed experiments. P.A., M.S.D., J.-H.H., A.H., S.T. carried out genomic comparisons and phylogenetic reconciliation. S.P.P. did the environmental sampling and populations structure analysis. All authors contributed to writing and editing the manuscript.

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