

OPINION

The genomic code: inferring Vibrionaceae niche specialization

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Abstract | The Vibrionaceae show a wide range of niche specialization, from free-living forms to those attached to biotic and abiotic surfaces, from symbionts to pathogens and from estuarine inhabitants to deep-sea piezophiles. The existence of complete genome sequences for closely related species from varied aquatic niches makes this group an excellent case study for genome comparison.

The class γ -Proteobacteria contains several medically important families, including the Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. The family Vibrionaceae consists of three genera, *Vibrio*, *Salinivibrio* and *Photobacterium*, with 44 species currently classified as *Vibrio*¹. The purpose of this article is to offer an opinion on what can be learnt from the available genome sequences of five Vibrionaceae species and how comparative genome analysis can help us to understand the complex lifestyles of these aquatic bacteria.

The Vibrionaceae: basic characteristics

To date, six genome sequences are available from the Vibrionaceae: *Vibrio cholerae* N16961, *Vibrio parahaemolyticus* RIMD 2210633, *Vibrio vulnificus* strains YJ016 and CMCP6, *Vibrio fischeri* ES114 and *Photobacterium profundum* SS9 (REFS 2–6) (TABLE 1). *V. cholerae* N16961 and *V. fischeri* ES114 have the smallest genomes at 4.0 Mb and 4.3 Mb, respectively, and *P. profundum* SS9 has the largest genome, at 6.4 Mb^{2–6} (TABLE 1). *Vibrio* species can colonize fish and marine invertebrates, can be associated with plankton and algae, and have the ability to form biofilms on biotic and abiotic surfaces, which has an essential role in their environmental persistence¹ (FIG. 1). *V. cholerae* is the aetiological agent of cholera, and so far, is the most widely reported *Vibrio* species. *V. parahaemolyticus* is the leading cause of gastroenteritis associated with the consumption of contaminated seafood, and

V. vulnificus causes fulminant septicaemia in susceptible hosts and has one of the highest mortality rates of any known pathogen^{4,7}.

V. fischeri is an aquatic free-living bacterium that colonizes the light organ of the squid *Euprymna scolopes*, forming a symbiotic relationship⁸ (FIG. 1). Finally, *P. profundum* is a deep-sea piezophile (an organism that thrives at high pressures) and the available genome sequence provides us with a unique insight into the evolution of bacteria in this particular environment⁶.

Understanding the Vibrionaceae

Close analysis of the distribution of clusters of orthologous groups (COGs) among the sequenced Vibrionaceae highlights both common and niche-specific signatures (FIG. 2a). The genes required for the tricarboxylic acid (TCA) cycle and glycolysis to support both respiratory and fermentative growth are present on all the sequenced genomes examined. However, there are some species-specific differences, such as the presence of genes for the respiratory nitrate reductase complex in *V. cholerae*. A study by Mrázek and colleagues proposed that *V. cholerae* and *V. vulnificus* prefer anaerobic growth by fermentation whereas *V. parahaemolyticus* is well adapted for both respiratory and fermentative growth⁹. This was based on determining the number of predicted highly expressed (PHX) genes involved in these functions, and might reflect the organism's predominant lifestyle⁹.

Genes encoding complete phospho-transfer system (PTS) phosphoryl transfer chains and multiple types of PTS permeases are present on each genome, indicating the ability to use a large number of sugars. The genomes also contain a large number of genes encoding gene products that are involved in transcription, motility and signal-transduction mechanisms, when compared with other aquatic, host-adapted and terrestrial bacteria (FIG. 2a). The large number of signal-transduction genes might reflect the requirements for interaction with their hosts either as pathogens or symbionts.

V. fischeri possesses a higher percentage of genes encoding proteins involved in cell wall and membrane biogenesis compared with the other *Vibrio* species (FIG. 2a). Studies on the interaction of *V. fischeri* with *E. scolopes* have identified peptidoglycan derivatives and lipopolysaccharide as triggers of specific development events in the host⁸. *P. profundum* has a higher number of open reading frames (ORFs) encoding enzymes involved in replication, recombination and repair than found in most other bacteria; this characteristic is also seen in another piezophile, *Shewanella oneidensis*, suggesting a pressure-sensitive role for these enzymes.

An interesting physiological feature of many *Vibrio* species is their extremely short replication time, in some cases less than 9 minutes¹⁰. This is reflected in their genomes by the presence of a higher than average number of rRNA and tRNA genes (FIG. 2b,c). The majority of the sequenced bacterial genomes contain between one and seven 16S rRNA genes; among the sequenced Vibrionaceae, the number ranges from eight in *V. cholerae* to fifteen in *P. profundum* (FIG. 2b; TABLE 1).

A short replication time could facilitate colonization where a large number of cells could overwhelm the host in a short period of time and give the bacterium a fitness advantage by outcompeting other bacteria. A short generation time also requires a large number of tRNA genes to translate the mRNAs being sent from the ribosomes. Consistent with this, the Vibrionaceae encode more tRNA genes (112–169) than the average for bacteria (50) (FIG. 2c; TABLE 1). Interestingly, the tRNA genes are hot spots for the insertion of novel

Table 1 | **Genome composition among members of the family Vibrionaceae**

Vibrionaceae species	Genome size (Mb)	% GC	Proteins	Origin (kb)	Number of tRNAs	Number of rRNAs	Number of integrons	Refs
<i>Vibrio cholerae</i> N16961	4.03							2
Chromosome 1	2.96	47	2,742	7	94	8	0	
Chromosome 2	1.07	47	1,093	100	4	0	SI	
<i>Vibrio parahaemolyticus</i> RIMD 2210633	5.17							4
Chromosome 1	3.29	45	3,080	3,287	112	10	1	
Chromosome 2	1.88	45	1,752	0	14	1	0	
<i>Vibrio vulnificus</i> YJ016	5.26							3
Chromosome 1	3.35	46	3,259	3,353	100	8	SI	
Chromosome 2	1.86	47	1,696	1,857	12	1	0	
Plasmid (pYJ016)	0.049	45	69	29	0	0	0	
<i>Vibrio vulnificus</i> CMCP6	5.12							(Ref. Seq. NC_004459)
Chromosome 1	3.28	46	2,926	1,006	98	8	SI	
Chromosome 2	1.84	47	1,562	1,276	13	1	0	
<i>Vibrio fischeri</i> ES114	4.28							5
Chromosome 1	2.91	39	2,575	6	108	11	0	
Chromosome 2	1.33	37	1,172	0	11	1	1	
Plasmid (pES100)	0.045	38	55	Unknown	0	0	0	
<i>Photobacterium profundum</i> SS9	6.4							6
Chromosome 1	4.09	42	3,416	4,085	145	14	0	
Chromosome 2	2.24	41	2,008	0	32	1	0	
Plasmid (pPBPR1)	0.080	44	55	31	0	0	0	

SI, superintegron.

DNA, and comparative genome analysis revealed preferential insertion of novel DNA at three tRNA sites among the Vibrionaceae: tRNA^{Ser}, tRNA^{Met} and tmRNA, with tmRNA being a generally popular insertion site for novel DNA in bacteria^{11–13}.

Two is better than one

An interesting although not unique feature in the genetic organization of the Vibrionaceae is the presence of two chromosomes of unequal size^{14,15} (TABLE 1). This feature is also found in other Proteobacteria, with another example from the γ -Proteobacteria being *Pseudoaltermonas haloplanktis*. In the Vibrionaceae, the larger chromosome, chromosome 1, generally carries most of the essential genes whereas the smaller chromosome, chromosome 2, carries more species-specific genes. Many studies have focused on this distribution of functional genes between the large and small chromosomes^{2,4,16,17}.

The presence of genes encoding proteins involved in several essential metabolic and regulatory pathways on chromosome 2 demonstrates that this smaller chromosome is essential for growth and viability^{2–4}. Indeed,

it has been proposed that a two-chromosome genome structure is advantageous to vibrios under the specific environmental conditions that they encounter in their life cycles, and might contribute to the environmental diversity in the genus^{14,18}. It has been proposed that chromosome 2 might have been acquired as a megaplasmid, probably before the diversification of the Vibrionaceae^{2,19}.

The small chromosome has probably evolved an as-yet-undefined specialized function leading to selective pressure to maintain independent replication^{2,14,19}. The presence of two replicons might also be an important factor in the ability to replicate rapidly and therefore add to the evolutionary fitness of these aquatic bacteria. Although the origins of replication (*ori*) of both chromosomes are different, they share common initiation factors and initiate replication synchronously^{19,20}.

Our analysis of the Vibrionaceae using the **Artemis Comparison Tool** (ACT) revealed that the evolution of their genome structure is marked by several intra- and interchromosomal rearrangements, but the overall gene content and position in

chromosome 1 is better conserved than in the smaller chromosome 2 (REFS 21,22) (FIG. 3). There is some conservation of gene order and sequence homology between chromosome 2 of *V. vulnificus* YJ016 and *V. parahaemolyticus* RIMD 2210633, however, limited sequence similarity was identified between the other species examined at chromosome 2 using ACT (FIG. 3).

Stress in the aquatic environment

Survival in any particular ecosystem requires a microorganism to be equipped with a battery of adaptive response mechanisms to meet demands such as nutrient limitation, UV stress, temperature fluctuations, protozoan predation, viral infection and changes in salinity. Many of these responses are mediated through quorum sensing, a global phenomenon first identified in luminescent *V. fischeri* and *Vibrio harveyi*²³. The signalling systems identified among the Vibrionaceae are divergent and respond to different environmental stimuli that are related to the interaction between a specific species and its environment or host²⁴. The LuxI/R system, the paradigm for quorum sensing

in Gram-negative bacteria, along with the AinSR and LuxP/QS systems, have been identified in *V. fischeri* but are not found in all the Vibrionaceae²⁵. The *V. harveyi* and *V. parahaemolyticus* genomes encode three common systems that respond in an independent manner to stimuli but converge on a single regulatory protein, LuxO²⁶. *V. cholerae* has an as-yet-uncharacterized system in addition to the LuxP/QS and CqsSA systems identified in *V. harveyi*, and *V. vulnificus* only has the LuxP/QS system. As well as encoding CqsSA and LuxS homologues, *P. profundum* seems to encode a new quorum-sensing system, which has approximately 35% sequence identity with the LuxMN and AinSR systems of *V. harveyi* and *V. fischeri*, respectively. These quorum-sensing systems function as coordinate regulators of a wide range of phenotypes at different cell densities and mediate the response of the Vibrionaceae to environmental stress, as well as regulating such phenotypes as biofilm formation and virulence^{27–30}.

Biofilm formation and phase variation have a crucial role in niche persistence of the *Vibrio* species in otherwise deleterious environments^{31,32}. Reversible phase variation between the rugose and smooth colony variants is important for the survival of *Vibrio* species in natural aquatic habitats. Epidemic strains of *V. cholerae* switch from the smooth to the rugose phase more frequently than environmental isolates, and this phase transition increases the resistance of the organism to osmotic, acid and oxidative stress, and enhances its capacity to form a biofilm^{33–36}. Another adaptive advantage of biofilm formation was shown by Matz and co-workers, who found that biofilms enable *V. cholerae* to survive protozoan grazing whereas free-living *V. cholerae* were eliminated³⁷. Gene clusters mediating biofilm formation have previously been identified in *V. cholerae* (*eps*) and *V. fischeri* (*syp*)^{36,38}. Whereas the *eps* gene cluster is species-specific, homologues of the *V. fischeri* *syp* gene cluster are also found in *V. parahaemolyticus*, *V. vulnificus* and *P. profundum*, although the arrangement and content of this cluster can vary³⁸.

Quorum sensing and biofilm formation are broad strategies for survival, however these bacteria have evolved more specific approaches to stress and survival. The *cadBAC* operon, which encodes a lysine decarboxylase, a lysine/cadaverine antiporter and a regulator, respectively, is an essential component in the acid-tolerance response^{39,40}. The *cadBAC* operon is located on chromosome 1 of each of the *Vibrio* species but is absent from *P. profundum*.

Another operon, *sspABC*, which is known to function as part of the acid-tolerance response, is found on chromosome 1, as are multiple homologues of the heat-shock protein DnaK, in all the species examined. It is possible that as a result of its deep-sea niche, *P. profundum* does not require a robust acid-tolerance response in the same way as the other Vibrionaceae do. A copy of the gene encoding the GroEL heat-shock protein is located on each chromosome, with the exception of *V. parahaemolyticus*, which has only a single copy on chromosome 1. At least eight other heat-shock proteins are dispersed throughout each of the genomes, indicating that this response is a major part of the survival mechanisms of these bacteria.

All the Vibrionaceae encode several cold-shock proteins, however, *P. profundum* is unusual in that it encodes twice as many cold-shock proteins as the other species, which might aid the bacterium in its extreme environmental niche. A recent study by Smith and Oliver suggested that the natural role for haemolysin (*vvhA*) in *V. vulnificus* could be in osmoregulation and the cold-shock response⁴¹. Homologues of the *vvhA* gene are found on all the sequenced Vibrionaceae genomes and therefore might have a similar role in these species.

Osmotic stress is an aquatic and host-related stress and it presents a niche-specific challenge to each species. Many bacteria mediate their response to osmotic pressure through the synthesis and uptake of compatible solutes (osmolytes). In particular, estuarine ecosystems, which are positioned at the interface between freshwater systems and the sea, present an additional challenge to their inhabitants, which must continually adapt to temporal and spatial fluctuations in salt concentration. *V. cholerae*, *V. parahaemolyticus* and *V. fischeri* all possess an *ectABC* operon that synthesizes ectoine, and a *bcct* gene responsible for the transport of betaine, choline and carnithine, which have all been shown to function as osmolytes in *V. cholerae*^{42,43} (FIG. 4).

V. parahaemolyticus and *V. fischeri* possess an *Escherichia coli*-like *proVWX* operon encoding proteins responsible for the uptake and transport of proline and glycine betaine, which is also reported to be capable of the transport and uptake of ectoine in *E. coli* (FIG. 4). In *V. fischeri*, the *ectABC* and *E. coli*-like *proVWX* operons are found on chromosomes 2 and 1, respectively, whereas in *V. parahaemolyticus*, the *ectABC*, *bcct* and *E. coli*-like *proVWX* genes are colocalized on chromosome 1 (FIG. 4).

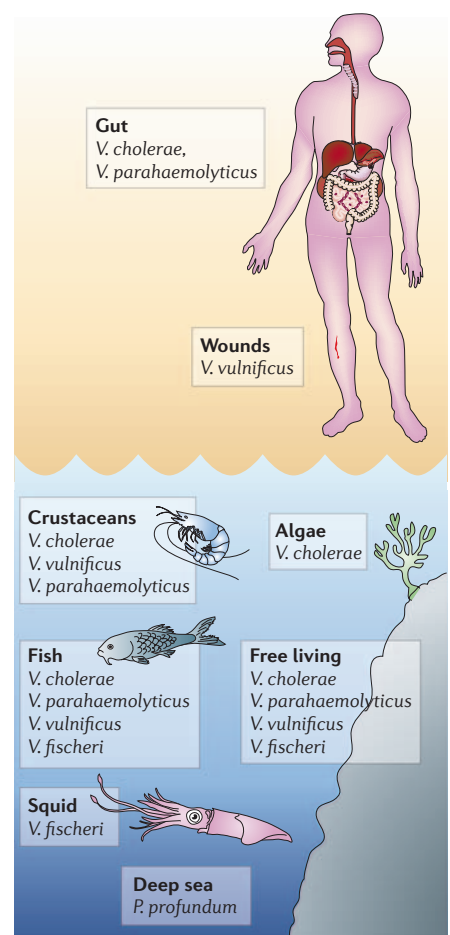


Figure 1 | Niche specialization of the Vibrionaceae. Although all Vibrionaceae are aquatic, they can be found in a wide range of niches. The four *Vibrio* species shown can colonize fish and marine invertebrates and can be associated with plankton and algae. *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* infect humans. *Vibrio fischeri* colonizes the luminous organ of the squid *Euprymna scolopes*, forming a symbiotic relationship. *Photobacterium profundum* is a deep-sea piezophile.

On chromosome 2 of *V. parahaemolyticus*, *V. vulnificus* and *P. profundum*, the *betABI* genes are located next to a *Pseudomonas*-like *proVWX* gene cluster (FIG. 4). The genomic diversity of the osmotolerance response in the Vibrionaceae is complemented by a cooperativity phenomenon in microbial communities, whereby compatible solutes synthesized by a particular species are secreted into the close environment where they can be scavenged by other species⁴². The significance of the unique gene clustering in *V. parahaemolyticus* has yet to be determined, but it suggests an integral role for these genes in withstanding fluctuating salinity and niche adaptation of the species (FIG. 4).

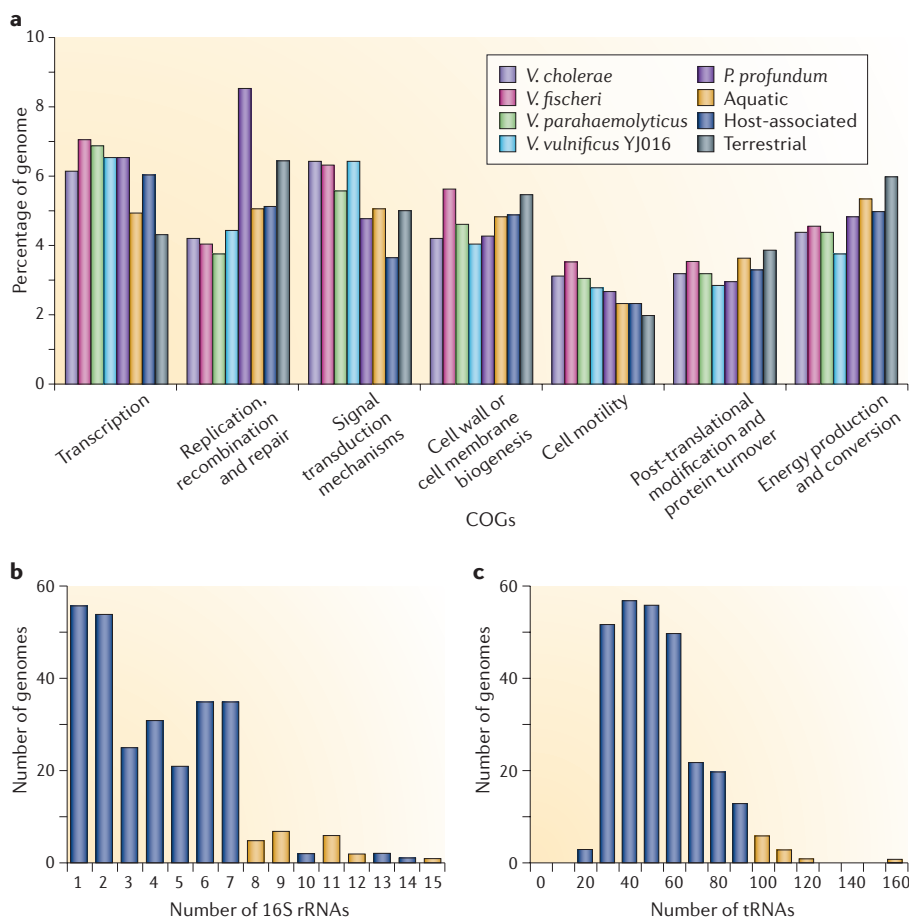


Figure 2 | **Genome mining to define Vibrionaceae characteristics.** **a** | Distribution of clusters of orthologous groups (COGs) among the Vibrionaceae in the wider context of aquatic, host-associated and terrestrial species. The data available at NCBI were used to generate a distribution chart in which the gene content for any particular function can be compared across the genus and in the context of the Proteobacteria and bacteria as a whole. **b, c** | Distribution of rRNA and tRNA genes among the sequenced bacterial genomes. The sequenced vibrios are indicated in yellow. The y-axis represents the number of sequenced genomes containing a particular number of rRNA or tRNA genes (x-axis).

The toxin, pilin and chitin connection

The two main virulence factors of *V. cholerae*, cholera toxin (CT), which is encoded on the filamentous cholera toxin phage (CTX ϕ) and causes a profuse watery diarrhoea, and the toxin co-regulated pilus (TCP), which is an essential intestinal colonization factor and the host receptor for CT, were both acquired by a subset of isolates by horizontal gene transfer (HGT)^{44,45}. In the aquatic environment, *V. cholerae* has been found in association with zooplankton and phytoplankton, on the chitinous exoskeletons and moults of copepods (crustaceans) and in the mucilaginous sheaths of blue-green algae^{46–49}. The mannose-sensitive haemagglutinin (MSHA), which is encoded by all the sequenced Vibrionaceae genomes, is involved in *V. cholerae* adherence to zooplankton⁵⁰. Recent studies have described a role for a

chitin-binding protein, ORF **VCA0811**, from *V. cholerae* in attachment to both the chitinous exoskeleton of zooplankton and human epithelial cells, by binding to the sugars present on both surfaces^{51,52}. This protein is found in the genomes of all sequenced Vibrionaceae and might also be involved in the environmental persistence of these bacteria.

V. fischeri exists naturally either in a free-living planktonic state or as a symbiont of the luminescent bobtail squid, *E. scolopes*⁵³. *V. fischeri* provides the source of luminescence, which the squid can use for camouflage, protection against predators, and which might also be involved in mating⁸. The bacteria only luminesce in this symbiotic state in the light organ and not when they are free living. The interaction of *V. fischeri* with the light organ of squid

involves several genes, including *mot*, *fla*, *flr* and *rpoN*⁸. Colonization is aided by the secretion of a mucoid substance above the pores of the light organ, which traps the *V. fischeri*, and MSHA is required for subsequent colonization^{54,55}.

Although, at first glance, the human pathogen *V. cholerae* and the squid symbiont *V. fischeri* have markedly different lifestyles, there are suggestive similarities between both species. In particular, the abundance of chitinase genes in their genomes reflects the high degradative ability attributed to vibrios⁵⁶. Chitin is a biopolymer of *N*-acetylglucosamine and, after cellulose, is the most abundant carbohydrate polymer; it is present in large quantities in the sea, being a constituent of the exoskeletons of crustaceans and zooplankton. Chitin induces TCP production, which, along with MSHA, allows *V. cholerae* to colonize the exoskeletons of crustaceans and zooplankton^{57,58}. *V. cholerae*, in addition to *V. fischeri*, can use chitin as a carbon and nitrogen source. Homologues of TCP were identified in the *V. fischeri* genome sequence⁵. Chitin might therefore have a similar role in *V. fischeri*, stimulating TCP-mediated biofilm formation or perhaps even facilitating colonization of the squid.

The diversity of TCP-mediated bacteria–host interactions in the aquatic niche has led to speculation on the role of CT in the environment^{59,60}. CT might function as an osmoregulator for crustaceans by the removal of salts from the cell by its intrinsic function. Briefly, CT increases Cl[–] secretion and reduces Na⁺ absorption, which could be advantageous to the crustacean as it moves into environments of increasing salinity⁶⁰. As *V. cholerae* is associated with copepods, *V. cholerae* might establish a symbiotic relationship with these crustaceans, obtaining a suitable place (chitinous exoskeleton) to attach and feed, and providing the host with a powerful osmoregulator (CT). Homologues of the CTX prophage were identified in the sequenced genome of *V. fischeri*, however this prophage did not carry the *ctxAB* genes⁵. CTX prophage lacking the *ctxAB* genes were also observed in *V. cholerae*, indicating the presence of a precursor CTX phage that acquired the CT genes from a still-unknown source⁶¹.

Another pathogenicity factor in *V. cholerae* is neuraminidase, which is encoded by the *nanH* gene on *Vibrio* pathogenicity island-2 (VPI-2)⁶². Neuraminidase cleaves mucin from intestinal cells, unmasking GM1 gangliosides, the receptors for CT, and releasing sialic acid, a carbon source^{63,64}.

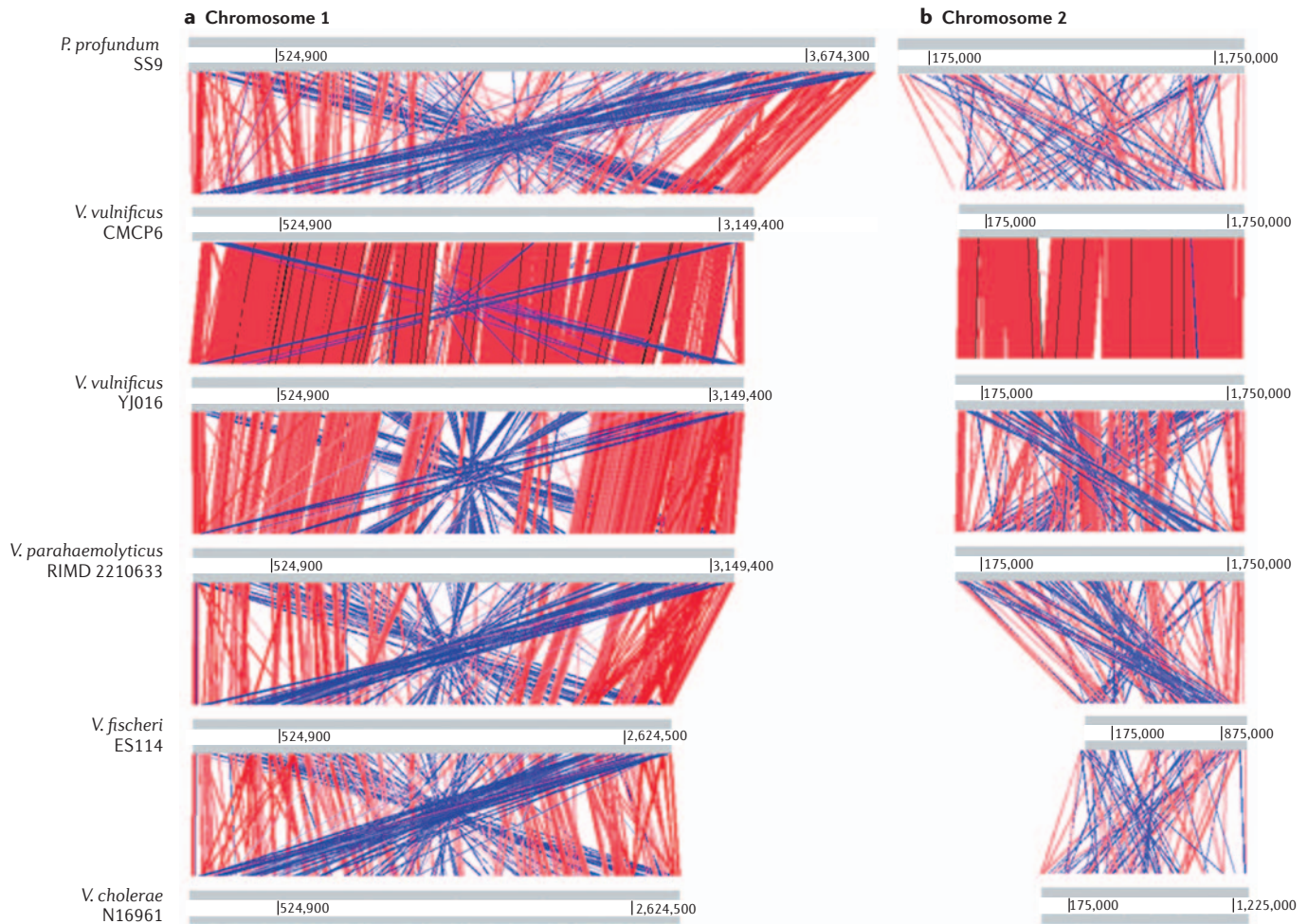


Figure 3 | Linear pairwise genome comparison of the Vibrionaceae. The analysis was performed using the Artemis Comparison Tool (ACT). Regions with similarity are highlighted by connecting red or blue lines between the genomes; red lines indicate homologous blocks of sequence and blue lines indicate inversions. The grey bars represent the forward and reverse strands. The gene order and orientation is highly conserved between both *Vibrio vulnificus* strains and *Vibrio parahaemolyticus* RIMD 2210633, as

seen by the large red blocks connecting both genomes, whereas the gaps are indicative of unique DNA. This is true to a lesser degree between *V. parahaemolyticus* RIMD 2210633 and *Vibrio fischeri* ES114, and *V. vulnificus* YJ016 and *Photobacterium profundum* SS9. For the purpose of this genome comparison, we used the reverse complement of GenBank file AE016795 containing the sequence of *V. vulnificus* strain CMCP6, whereby the origin was placed at coordinate 1.

Pathogenic isolates of *V. cholerae* have been found in association with the green algae *Anabaena* spp., which have a mucilaginous sheath^{47,65,66}. *V. cholerae* isolates encoding neuraminidase could have a selective advantage in the ability to use mucilage as a carbon source in their natural environment. The *nanH* gene has a limited distribution among the sequenced Vibrionaceae and has so far only been found in *V. cholerae* O1 serogroup strains⁶⁷. Although lacking the gene that would enable them to scavenge sialic acid from the environment, the other sequenced vibrios all encode the genes required for sialic acid synthesis (*neuABCD*), which are absent from *V. cholerae*. The significance of this is yet to be understood.

An additional connection between toxin, pilin and chitin is the recent discovery that

V. cholerae isolates are naturally competent to take up DNA in the presence of chitin⁶⁸. Both CT encoded on CTX ϕ and TCP encoded on *Vibrio* pathogenicity island-1 (VPI-1) were acquired by HGT, probably by transduction. Transformation in the natural environment offers another mechanism for *V. cholerae* to acquire novel DNA and might indicate a possible mechanism of DNA acquisition among the Vibrionaceae in general.

Emerging pathogens with unique genes

In this section, we will examine further the role that HGT and recombination has played in niche specialization by analysing within-species diversity in gene content. It is important to note that within the same species, different strains can behave differently, for example in *V. cholerae* only 0.8%

of the strains isolated in a cholera-endemic area encoded TCP and CTX ϕ ⁶⁹. In fact, the majority of serogroups, with the exception of the O1 and O139 groups, do not encode CTX ϕ or TCP. Different ecotypes can exist within the same species and those isolates that carry CTX ϕ and TCP belong to one ecotype, which might preferentially associate with humans and crustaceans, and other ecotypes might carry traits that allow them to enter other states (for example, free living or associated with other animals or plants).

Studies examining the presence of strain-specific DNA among pathogenic *V. cholerae* isolates have shown the acquisition of several regions that are specific to pandemic isolates^{44,45,62,70,71}. The emergence of a novel epidemic *V. cholerae* O139 serogroup in 1992 resulted from the acquisition of the O139

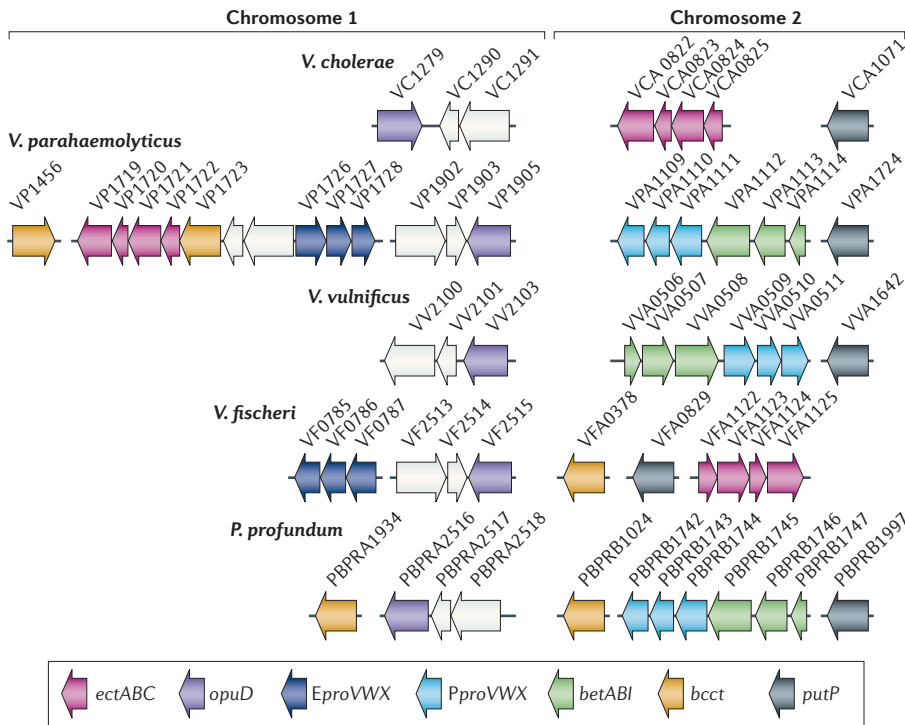


Figure 4 | **Genomic appraisal of osmoregulation among the Vibrionaceae.** Osmoregulation and adaptation to fluctuating salinity present niche-specific challenges to marine bacteria. This is reflected in the diversity and complexity of their osmotic stress response systems. The two synthesis systems, ectoine (*ectABC*; pink) and glycine betaine (*betABl*; green), are only found together in *Vibrio parahaemolyticus*. An either/or situation applies among the other Vibrionaceae. The direction of the arrows indicates the strand on which the genes are found, where forward arrows represent genes on the positive strand of the genome. The diversity in both gene content and arrangement of the osmoregulatory systems of the Vibrionaceae is clearly evident. *bcct*, Betaine carnithine choline transporter; *EproVWX*, *Escherichia coli*-like glycine betaine/proline transporter; *opuD*, transporter BCCT family; *PproVWX* *Pseudomonas*-like glycine betaine/proline transporter; *putP*, sodium/proline symporter.

gene cluster, and a capsule polysaccharide and an integrative conjugative element^{48,69}. A type III secretion system (TTSS) was identified in the genome sequence of a clinical *V. cholerae* CT-negative isolate, which imparts an alternative pathogenesis strategy⁷². Examination of *V. cholerae* environmental isolates by subtractive hybridization also uncovered several additional regions absent from pandemic isolates, which might provide interesting data on niche specialization⁷³.

Unlike *V. cholerae*, which causes secretory diarrhoea by producing CT, *V. parahaemolyticus* can cause gastroenteritis associated with inflammatory diarrhoea^{74,75}. In recent years, outbreaks of *V. parahaemolyticus* infection have increased worldwide and in regions with high seafood consumption, *V. parahaemolyticus* causes over half of all food poisoning outbreaks of bacterial origin⁷⁵. One of the main *V. parahaemolyticus* virulence factors is the thermostable direct haemolysin (TDH). TDH is a pore-forming protein that might contribute to the

invasiveness of the bacterium. Sequencing of the *V. parahaemolyticus* RIMD 2210633 serogroup O3:K6 genome identified two TTSSs, TTSS-1 on chromosome 1 and TTSS-2 on chromosome 2, encoded within a pathogenicity island that also encodes TDH⁴. TTSS-1 is present in both clinical and environmental isolates whereas TTSS-2 is only present in clinical isolates. Until 1995, several different serogroups were associated with outbreaks of gastroenteritis caused by *V. parahaemolyticus*, however, in 1996 a new serogroup emerged, O3:K6, which has since spread worldwide and is the main cause of seafood-borne bacterial-associated gastroenteritis⁷⁶. Strains of the new O3:K6 serogroup all encode the TTSS-2 gene cluster and TDH⁴.

Bioinformatics analysis of the sequenced *V. parahaemolyticus* genome identified seven regions that had the characteristics of genomic islands¹¹. Molecular analyses of the distribution of these genomic islands among pre- and post-1995

pandemic isolates found that they were mainly present in the new O3:K6 pandemic strains¹¹. These data suggest that new pandemic *V. parahaemolyticus* O3:K6 isolates have, in a short timeframe, acquired large regions of novel DNA and that their acquisition might have aided the pandemic spread and pathogenesis of this organism in humans¹¹. Similar to *V. cholerae*, a subset of *V. parahaemolyticus* isolates have obtained unique regions of DNA that allow them to colonize humans.

The mechanism by which *V. vulnificus* is pathogenic to humans depends, to a large extent, on host susceptibility, and this bacterium is predominantly an opportunistic pathogen⁷. Human infections occur generally after consumption of contaminated raw oysters or wound infection from seawater or contaminated fish⁷. Multiple virulence factors, including the capsular polysaccharide (CPS), and iron availability in the host, as well as a short generation time, contribute to the highly invasive nature of *V. vulnificus*^{7,77,78}. Host susceptibility has a crucial role in *V. vulnificus* disease progression; hepatitis, haemochromatosis and an impaired immune system can result in lethal septicaemia⁷. Among *V. cholerae* and *V. parahaemolyticus*, pathogenic isolates are clearly defined by the presence of CT and TDH, respectively, however, among *V. vulnificus* isolates host susceptibility seems to be a defining factor for virulence.

Several clinical biomarkers have been identified for *V. vulnificus*, such as allelic variants of 16S RNA, *viuB* encoding a vibriobactin-utilization protein, and ORF VV0401 encoding a conserved hypothetical protein (**VV0401**), and all clinical isolates are encapsulated^{131,79-81}. Group 1 CPS allelic variants are differentially distributed among clinical and environmental strains of *V. vulnificus* and the role of CPS in virulence has been clearly shown³¹. Comparative genomic analysis of the two sequenced *V. vulnificus* strains YJ016 and CMCP6 identified strain-specific genomic islands, and most of these regions were absent from a range of clinical and environmental isolates examined¹². To date, regions associated predominantly with clinical isolates have not been identified.

When prophages, integrons and genomic islands are included in the equation, over 500 kb of novel DNA unique to subsets of strains for each species has been discovered^{11,12,16,70,72}. Although many of the regions acquired by the Vibrionaceae have yet to be characterized and their functions defined, it is likely that they have a fundamental

role in the adaptation of these species to, and survival within, their ecosystem. As mentioned previously, each of the sequenced genomes contains an intron of diverse size (range 36 to 219 cassettes) and content. *V. vulnificus* strains YJ016 and CMCP6 have 88 and 114 cassette types, respectively, but only share 34 cassettes⁸². The precise role of these elements in the evolution of vibrios remains unknown, but their contribution to genome diversity is significant. The ability of the Vibrionaceae to acquire genetic material from the rich ocean resources (phage DNA and free DNA) by transduction and transformation provides them with a dynamic means of ecological adaptation within a species.

Life under pressure

P. profundum is a piezophilic psychrophile and has been isolated from a variety of deep-sea environments in the Pacific ocean, where it can thrive on the sea floor under pressures of 15,000 psi⁸³. Like the other members of the Vibrionaceae family, *P. profundum* can be free living or associated with fish.

At first sight, it might seem that a bacterium adapted to this extreme environment should carry numerous traits that allow it to survive, but it has been found to be less complex than might be expected⁶. Many of the genes required for survival at high pressures are also found in shallow-water microorganisms, indicating that high-pressure selection has not required the evolution of dramatically different lineages of life⁶. This is further exemplified by the degree of synteny between the *P. profundum* genome and the other sequenced Vibrionaceae (FIG. 3).

It could also be argued that differential regulation of common gene sets could be responsible for adaptation to high-pressure environments⁶. *P. profundum* SS9 responds to changes in temperature and pressure by inversely altering the expression of two genes encoding outer-membrane proteins and by changing its fatty-acid composition⁸⁴. Low pressure has also been shown to induce various stress responses in *P. profundum* SS9, reflected by the upregulation of chaperones and DNA repair enzymes¹⁶. These are common mechanisms used by deep-sea piezophiles to withstand fluctuations in their environment. Transport might also have an important role in adaptation to the high-pressure environment, reflected in the ability of piezophilic bacteria to choose between different transporters or metabolic strategies¹⁶. Comparison with moderate and non-piezophilic species of

Photobacterium have shed further light on this species. Campanaro and colleagues identified 171 coding sequences that were specifically absent or highly divergent in a pressure-sensitive strain when compared with *P. profundum* SS9 and another pressure-adapted strain¹⁶. This is yet another example of accelerated evolution by HGT leading to greater divergence and ecosystem diversity within species and genera.

Given the complexity of the deep-sea environment, characterization of these genes might give a greater insight into the adaptation of bacteria to environments of extreme temperature and pressure. In general, *P. profundum* contains more transposase genes (approximately 250) than the other species examined, whose numbers range from 1 for *V. fischeri*, to 48 for *V. vulnificus*. In the *P. profundum* genome, which is the largest of the sequenced Vibrionaceae, a higher proportion of repeat regions are present on chromosome 2 than on chromosome 1 (REF. 16).

Convergence and divergence

These aquatic organisms have evolved to occupy diverse niches in their ecosystems and to develop complex lifestyles, perhaps over hundreds of millions of years. The core genome of these bacteria encodes the proteins that allow them to exist in the aquatic ecosystem, and the unique DNA, in some cases acquired by HGT, has led each species to adapt to specialized environments (pathogens, symbionts, deep sea) with different degrees of success, which reflects the evolutionary age of these associations. Future research will uncover new and unexpected relationships between the Vibrionaceae and their environment, which could lead to a shift in the way pathogenesis is understood.

Several new *Vibrio* genome sequences are pending, including *Vibrio salmonicida*, *Vibrio alginolyticus*, *Vibrio lentus*, *Vibrio mimicus*, and *Vibrio splendidus*, which are found in diseased molluscs, shrimp and fish, as well as the deep-sea vent bacterium *Vibrio* sp. Ex25 and five additional *V. cholerae* strains. The release of these genomes will give us new insights into the elegant and parsimonious ways these bacteria have evolved niche diversification mechanisms. The challenge now is to develop systems capable of deciphering the vast array of data. To fully exploit the opportunity that now presents itself we need to pose new research questions and to design new experimental approaches that will unravel the genomic codes.

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Competing interests statement

The authors declare no competing financial interests

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