MICROBIAL BIODIVERSITY WITHIN THE VIBRIONACEAE

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1. The Vibrionaceae

1.1. A GENERAL DESCRIPTION

Vibrio takes its name from the Latin word *Vibrare*, meaning 'to wave'. Otto Müller first used the word *Vibrio* as a descriptor in the 18th century to describe bacteria with an elongated shape observed in culture (Rossello-Mora and Amann 2001). The family Vibrionaceae, first described by Véron (1965), resides within the γ-proteobacteria, one of the five subdivisions of the phylum Proteobacteria within the domain Bacteria.

All Proteobacteria are gram negative. This extremely metabolically diverse group of bacteria of medical and industrial importance has been divided into five subdivisions (α, β, γ, δ and ε) on the basis of 16S rRNA sequence data. The γ-Proteobacteria are divided into three major subgroups, denoted γ-1, γ-2, and γ-3. Members of the γ-1 and γ-2 subgroups contain sulfur producing photosynthetic bacteria and members of the family *Legionella*, respectively. The γ-3 subgroup consist of a "*potpourri*" of organisms, including oceanospirilla, many pseudomonads, the enterics, and the Vibrionaceae (Woese *et al*. 1985).

Vibrionaceae is one of 22 families within the 14 orders of the γ-Proteobacteria. Members of this family were first described as oxidase-positive, motile bacteria with polar flagella (Krieg and Holt 1984). More recent data demonstrate that all Vibrionaceae species are either straight or curved rods, with some species not having oxidase-positive phenotypes (*Vibrio metschnikovii, V. gasogenes, Photobacterium phosphoreum, P. angustum,* and some strains of *P. leiognathi* are oxidase-negative), and some being non-motile. Vibrionaceae currently contains six genera: *Vibrio*, *Allomonas*, *Enhydrobacter*, *Listonella*, *Photobacterium* and *Salinivibrio*. Each genera has its own distinguishing morphological and physiological characteristics (Krieg and Holt 1984). Phenotypic data used to define each genus are G/C content, the presence of sheathed polar flagella, requirement of sodium for growth, lipase activity, D-mannitol utilization, and sensitivity to vibriostatic compound 0/129 (Holt *et al*. 1994). The most recent online version (July, 2002) of Bergey's Taxonomic Outline lists 72 species of *Vibrio*, 7 of *Photobacterium*, 3 of *Listonella*, and one of the other three genera (Table 1). These facultative anaerobes are found almost exclusively in aquatic environments, and have G/C contents ranging from 38-66% (Krieg and Holt 1984).

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The genus *Enhydrobacter* is very unique among the Vibrionaceae. The only identified species in this genus, *E. aerosaccus*, forms gas-vacuoles, lacks luminescence, has a very high G/C content (66% compared to 38-51% for *Vibrio* and 40-44% for *Photobacterium* species). Furthermore, this species is non-motile and resistant to vibriostatic compound 0/129, making it quite an anomaly within the Vibrionaceae. Due to its very slow growth on unusual media (30-60 days), this species has not been studied to the extent of the other members of Vibrionaceae. In fact, a search in the Biological Abstracts and Science Citation Index only yields the paper in which *Enhydrobacter* is first described (Staley *et al*. 1987). Furthermore, the 16S rRNA has not been sequenced, and more work is necessary to determine the proper phylogenetic position of this distinct genus.

1.2. TAXANOMIC HISTORY

The advent of the polymerase chain reaction (PCR) and the resultant ease of sequencing DNA in the mid 1980's brought about a series of dramatic re-organizations within the Vibrionaceae. A complete review of all recent changes in species names within the Vibrionaceae is beyond the scope of this review; however, when examining the changes in genera within the past 16 years, it is interesting that only *Vibrio* and *Photobacterium* have not been deleted from the Vibrionaceae (Table 1).

Table 1. Changes in Vibrionaceae genera since 1984. Numbers in parentheses indicate the total number of described species in that genus.

1984[1]	**Current Family**	**July 2002**[2]	**Date Added**
Vibrio (20)	Unchanged	*Vibrio* (72)	-------
Photobacterium (3)	Unchanged	*Photobacterium* (7)	-------
Aeromonas (4)	Aeromonadaceae	*Allomonas* (1)	Kalina *et al*.(1984)
Pleisomonas (1)	Enterobacteriaceae	*Enhydrobacter* (1)	Staley *et al*. (1987)
		Listonella (3)	MacDonell and Colwell (1985)
		Salinovibrio (1)	Mellado *et al*. (1996)

[1]From Krieg and Holt (1984)
[2]From Garrity *et al*. (2002)

Between 1984 and the present, two families have been proposed and subsequently moved from Vibrionaceae. The first, *Shewanella*, was added together with *Listonella* by MacDonell and Colwell (1985). *Shewanella* was soon moved to the Alteromonadaceae based on 16S rRNA sequence analysis. *Colwellia* was then added to the Vibrionaceae (Demming *et al*. 1988) but subsequently relocated to the Alteromonadaceae. As more species are added to different families within the Vibrionaceae in the near future, the taxonomy will undoubtedly undergo many more significant changes.

2. Evolution of the Vibrionaceae

2.1. EVERCHANGING PHYLOGENY OF THE VIBRIOS

The family Vibrionaceae is one of the most well studied heterotrophic bacterial groups in marine ecosystems. They are important players in the upper surface waters, and inhabit a number of ecological niches, including fish intestinal tracts (Simidu *et al*. 1977), squid and fish light organs (Ruby and Nealson 1976; McFall-Ngai 2000), and are found as crustacean ectosymbionts (Roszak and Colwell 1987). Because of their broad ecological distribution and importance in marine community structure, their taxonomic identification has been extensively studied. Initially, the first phylogenetic accounts of vibrios were completed using phenotypic and genotypic analyses. These studies relied on DNA-DNA hybridzation, DNA-rRNA hybridization, enzymatic activity, restriction fragment length polymorphism analysis (RFLP), physiological growth at various temperatures, carbon source utilization, protein fingerprinting, and substituted amino acids to name a few (Baumann and Baumann 1977, Baumann *et al*. 1980a, 1983, 1984; Baumann and Baumann 1981; Colwell 1984; Bryant *et al*. 1986a, 1986b; Martin-Kearley and Gow 1994; Montilla *et al*. 1995; Urakawa *et al*. 1997, 1999; Lunder *et al*. 2000). Although these methods produced initial taxonomic and phylogenetic information, the relationships among related taxa, particularly those that are now renamed, was still vague. According to Bergey's manual of determinative bacteriology, species of *Vibrio* increased from 5 in 1974 to 72 in 2002 (Shewn *et al*. 1974; Garrity *et al*. 2002). This increase was due to the fact that the family has undergone some drastic taxonomic revisions (with some of the species being placed in an entirely different genus) as well as the new discovery of psychrophilic and psychrotrophic species. In 1987, Carl Woese (Woese 1987) proposed the 3 kingdom tree of life, using 16S rRNA as a molecular chronometer for determining phylogenetic relationships. With the advancement of techniques for rapidly sequencing DNA and RNA, the revision of prokaryotic taxonomy (and in particular, the Archaea and Bacteria), has been a fundamental instigator for the revision of the Vibrionaceae family. Initial studies using DNA sequences primarily focused on the nuclear ribosomal RNAs (5S and 16S rRNAs) to delineate species among Bacteria and Archaea. From this, investigators interested in the identification and classification of *Vibrio* species used the small ribosomal subunit in a number of studies to confer previous proposed phylogenies. Some of these phylogenetic analyses conferred known relationships (*Photobacterium* and *Vibrio* as monophyletic; Baumann *et al*. 1980a; Urakawa *et al*. 1997) but also changed the relationships of others. The genus *Aeromonas* originally was within the Vibrionaceae, but after the work of Colwell and others (Bryant *et al*. 1986a; Colwell *et al*. 1986; Kita-Tsukamoto *et al*. 1993; Ruimy *et al*. 1994) using 5S and 16S rRNA sequence analyses, they confirmed that this genus was within the family Aeromonadaceae.

As for inter-species relationships within the genus *Vibrio*, several phylogenetic relationships have been elucidated with molecular data. *V. marinus* has been classified as significantly different from other species of vibrios analyzed (MacDonell and Colwell 1984; Steven 1990), and recently has been proposed to be renamed *Moritella marinus* based on 5S rRNA sequence and DNA-DNA hybridization studies. Also, the relationship between *V. cholerae* and *V. mimicus* has been ambiguous; the two species are found to be similar based on 16S DNA sequence analyses (Davis *et al*. 1981) as well

as a variety of phenotypic characters. Another example of incongruency is that of the relationship between *Vibrio anguillarum* and other related taxa. This species was previously placed as sister to *V. splendidus*, *Photobacterium angustum* and *V. metschnikovii* (Alsina and Blanch 1994), but has now been placed as sister to *V. damsela* and *V. ordalii* (Wiik *et al*. 1995). Again using the 16S rRNA as well as a combined analyses approach (genes and morphology) allows better resolution among taxa that have unclear associations (Figure 1). The 16S sequence data base for *Vibrio* species has also allowed the design of rRNA oligonucleotide probes to identify a number of pathogenic and free-living vibrios in the water column (Aznar *et al*. 1994). This technique can detect *Vibrio* cells from filtered water samples, and has initiated many studies examining the population and community structure that vibrios have on the total bacterioplankton ecology (Rehnstam *et al*. 1989). New, innovative approaches using additional morphlogical, DNA and RNA sequence data in combination will further the investigation of species divergence within the Vibrionaceae, and will help determine whether the taxonomy of many *Vibrio* species should be re-examined to provide a clearer picture of the diversity that this group represents (Figure 1).

2.2. THE EVOLUTION OF VIRULENCE IN MUTUALISTIC ASSOCIATIONS

Symbiosis among the Vibrionaceae occurs with many marine host species. Generally, vibrios colonize either crustacean (Bowser *et al*. 1981), mollusc (McFall-Ngai 2002), or fish (Wiik *et al*. 1995) hosts, either causing severe disease or death (Schiewe *et al*. 1981; Wiik *et al*. 1989; Toranzo and Barja 1990). Although a number of these pathogenic vibrios have common physiological attributes, it has always been a question of whether virulence or virulence factors (i.e., pathogencity islands) were common among these types of symbionts. Investigations assaying biochemical features (Lunder *et al*. 2000), iron sequestration (Tolmasky *et al*. 1985), and plasmid profiling (Sorum *et al*. 1990) have grouped many of the pathogens together, according to their specific hosts that they infect. Although this may provide a "common ground" for all species studied, 5S and 16S rRNA molecular data provide evidence that most of these alliances are not robust (Nishiguchi and Nair in press, Figure 1; Wiik *et al*. 1995) and the pathogenic species of *Vibrio* are not monophyletic. This is probably due to the fact that most phenotypic characters are more likely to place species or species groups according to their ecological niches; that is, phenotypic characters tend to reflect the type of habitat and the abiotic factors that influence the phenotype of that particular species or strain (Cohan 2002). One example of such behavior is found in the mutualistic association between vibrios and their sepiolid squid hosts (Nishiguchi 2001). Although this association is specific for 2 known *Vibrio* species (*V. logei* and *V. fischeri*; Fidopiastis *et al*. 1998), this particular symbiosis can be host specific (as seen in Indo-west Pacific populations of *Euprymna*; Nishiguchi *et al*. 1998, Nishiguchi 2002), or can be influenced by abiotic factors such as temperature (Nishiguchi 2000). Thus, virulence or virulence factors may be similar in gene homology (see section 4.2) but are not congruent with the phylogenic data based on 16S rRNA sequence (Nishiguchi, unpublished data, Figure 1).

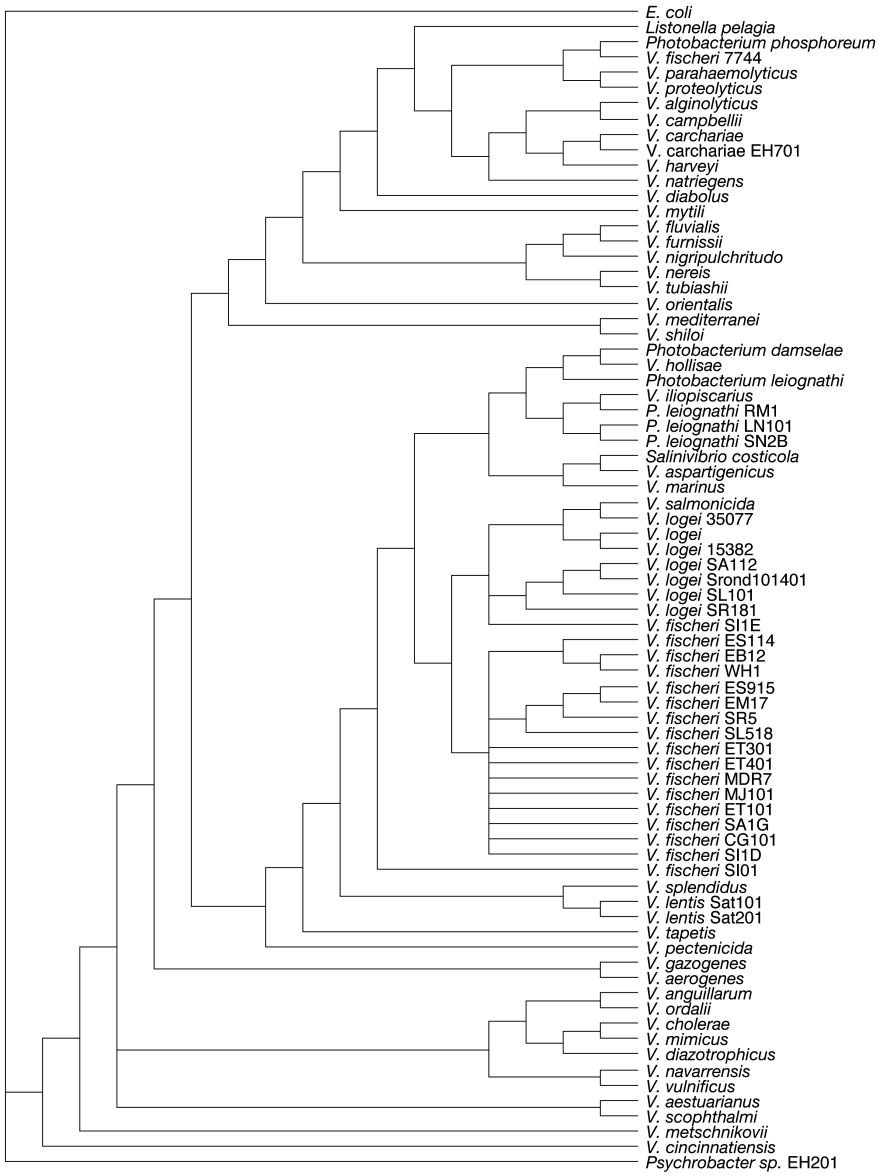

Figure 1. Strict consensus tree for 75 taxa of representative *Vibrio* strains and related species. Phylogenetic relationships are based on the complete 16S rRNA sequence from a number of Vibrionaceae representatives. Molecular data were analyzed using the direct optimization method (Wheeler 1996) as implemented in the computer program POY (Wheeler *et al*. 2002). A parameter space of two variables (gap/change ratio and transversion/transition ratio) was explored, as in Wheeler (1995). Transversion/transition ratios of 1 (equal weights) and 2 (transversions receive twice as much weight than transitions) were explored for these analyses. All species are designated with strain identification in the figure. For key, see http://mvar.nmsu.edu/nish/index.htm.

3. Ecology of the Vibrionaceae

3.1. DIVERSE HABITATS AND ASSOCIATIONS

As previously mentioned, members of Vibrionaceae are ubiquitous in the marine environment, with species found in hydrothermal vents (Raguenes *et al*. 1997), deep sea (Maruyama *et al*. 2000), open water (Eilers *et al*. 2000), estuaries, and marine sediments (Lee and Ruby 1994). Traditionally, the Vibrionaceae were thought to comprise a very large portion of bacterioplankton communities (ZoBell 1946); however, modern molecular techniques (such as fluorescent *in situ* hybridization) have shown these estimates to be wildly overstated (Eilers *et al*. 2000). More recent estimates range from 1 (Eilers *et al*. 2000) to 10 (Nishimura *et al*. 1995) percent of total cells, depending on the habitat. Although they comprise only a small percentage of total free-living bacteria in the marine environment, the importance of Vibrionaceae should not be underestimated. Recently, many studies have suggested that vibrios degrade some ecologically hazardous compounds, such as polycyclic aromatic hydrocarbons (Ramaiah *et al*. 2000), and are major decomposers of chitin in the ocean (Nagasawa and Terazaki 1987; Hedlung and Staley 2001). Vibrios have also been found in rivers (Kenzaka *et al*. 1998), and *V. cholerae* is known to inhabit freshwater environments (Baumann *et al*. 1980b). One species, *V. navarrensis* has even been found in sewage outfalls (Urdaci *et al*. 1991).

Free-living vibrios have not been taken directly from soil, but have been found in the gut of soil invertebrates (Byzov *et al*. 1996; Tret'yakova *et al*. 1996). Assuming the gut of these animals was colonized environmentally from soil (as opposed to vertical or horizontal colonization), there should be free-living vibrios in these habitats. Further evidence that vibrios are found in the free-living state in soil was determined by Hallmann *et al*. (1999) who found both *V. cholerae* and *V. fluvialis* in soil samples following the addition of chitin to a final concentration of 1% (w/w). Since many vibrios exist in a viable but non-culturable state (Colwell *et al*. 1985), they may not be detected in experiments which rely on traditional culture based techniques for data collection.

Perhaps ecologically and economically more important than the free-living species is the variety of parasitic and mutualistic relationships existing within the Vibrionaceae. The diversity of parasitic associations exhibited by vibrios is truly remarkable. The most common parasitic association is the attachment to the surface of fish and other animals as saprophytes. In fact, one of the traditional methods used to isolate strains of luminescent bacteria was to incubate a dead fish and inspect it for glowing colonies (Nealson and Hastings 1979). *V. cholerae*, the bacterium whose virulent form causes the disease cholera, attaches to chitinaceous zooplankton, such as copepods (Colwell 1996), using them as a vector and transport mechanism, causing spread of this deadly disease. Members of Vibrionaceae have also been shown to cause potentially lethal diseases in humans and fish (Farmer *et al*. 1985; Brayton *et al*. 1986; Kusuda and Kawai 1998; McCarter 1999). More recently, studies have shown *V. shiloi* to be a coral pathogen, producing toxins that inhibit photosynthesis and lyse zooxanthellae resulting in bleaching (Banin *et al*. 2000a, 2000b).

Mutualistic interactions with vibrios have also been widely studied. One of the most common places to find vibrios is the gut of marine animals. Since many Vibrionaceae species contain chitinase (Ramaiah *et al*. 2000), they most likely aide their host in digestion. Vibrios are also the symbionts in teleost fish light organs. In these mutualistic associations, the bacteria receive nutrients from the light organ, and light produced has a variety of functions, including prey attraction (the angler fish), predator evasion via light bursts or counterillumination (the pony fish), and interspecies communication (flashlight and pinecone fishes; Nealson and Hastings 1979). More recently, the sepiolid squid/*Vibrio* symbiosis has become a model system for the study of mutualistic interactions (Nishiguchi *et al*. 1998; McFall-Ngai 1999). In this association, the *Vibrio* symbiont inhabits a complex, bilobed light organ inside the squid mantle cavity. Light produced by the *Vibrio* symbiont aides the squid host as camouflage in the form of counterillumination (Jones and Nishiguchi, unpublished data). In return, the squid provides nutrients for the symbiotic *Vibrio*, resulting in a generation time of under 30 minutes (Boettcher and Ruby 1990; McFall-Ngai and Ruby 1998), which is much faster than the free-living state.

3.2. BIOLUMINESCENCE AND THE VIBRIONACEAE

Light production is a common characteristic of many members from the genera *Vibrio* and *Photobacterium*. Although species of other genera (including *Shewanella* and *Photorhabdus*) are also luminescent, *Photobacterium* and *Vibrio* species have been the most extensively studied (Meighen 1994; Bassler and Silverman 1995; Bourgois *et al*. 2001). In its purest form, the light emitting reaction involves the catalysis by the luciferase enzyme of a number of substrates, including reduced flavin mononucleotide ($FMNH_2$), a long chain fatty aldehyde (RCHO), and molecular oxygen (Meighen 1991).

Luminescence is created and regulated by the *lux* genes, of which only five are common to all light-producing bacteria. The genes *luxA* and *luxB* encode for the α and β subunits of luciferase, respectively. The *luxC*, *D*, and *E* genes encode proteins involved in the ultimate construction of the long chain fatty aldehyde from tetradecanoyl-ACP (Meighen 1994). Other species-specific genes include various regulatory genes (*luxR*, *I*, *L, M, N,* and *O*; see section 3.3 for discussion of quorum sensing), genes responsible for the modification of light wavelength or emission efficiency (*luxL* and *Y*), and genes whose function have not been clearly defined (*luxG* and *H*; Bassler and Silverman 1995). In addition to the many species-specific genes in the operon, gene order also appears to be species-specific, with the exception of *luxA* and *B*, which are always situated adjacent to each other in the operon. The *lux*-regulated light producing reactions are metabolically expensive. It has been estimated that up to 17-20% of total respiration in *V. fischeri* cells is used in luminescent reactions (Makemson 1986; Bourgois *et al*. 2001). This begs the question of why bacteria should produce light and what types of selective pressures have led to the development of such a genetically complex system.

Nealson and Hastings (1979) summarized hypotheses as to why this phenomenon occurs in some bacteria. The first hypothesis relates bioluminescence to its biological and ecological functions. Since most luminescent species are enterics, light production on a substrate would be advantageous if it attracted a predator whose gut would be colonized by the luminescent colony. Although some theoretical work has been

presented on the amount of bacteria required for a fish to detect a colony for consumption (Nealson and Hastings 1979), no known empirical tests have been conducted to assess this seemingly plausible argument.

The second set of hypotheses states that bioluminescence has a biochemical function, such as allowing the cell to benefit from photochemical reactions. Recently, Czyz *et al*. (2000) proposed that bioluminescence functions to repair damaged DNA. In this study, the authors noticed that UV irradiation-sensitive mutants of *V. harveyi* tended to lack a functional luminescence system. When these mutants were incubated in the dark following UV irradiation, their survival was significantly depressed compared to non-mutants incubated under the same conditions. Another biochemical possibility would be the use of the luminescence system to aide in the re-oxidation of excess reducing equivalents in the cell when the respiratory system nears saturation in near anaerobic environmental conditions (Makemson and Hastings 1986; Bourgois *et al*. 2001).

McElroy and Seliger (1962) proposed a separate hypothesis associated with the evolution of bacterial luminescence that is similar to many of the biochemical function hypotheses. This hypothesis states that bioluminescence first evolved as a detoxification mechanism via the reduction of molecular oxygen. Watanabe *et al*. (1993) suggested that prior to the evolution of the *lux* genes responsible for the formation of the long-chain aldehyde, luciferase may have acted to detoxify H_2O_2. This hypothesis is discussed in detail by Timmins *et al*. (2001). Although the hypotheses dealing with a biochemical function of light-production are intriguing, most (with the exception of Czyz *et al*. 2000) fail to provide a functional role of the light produced. More research into the biological and ecological advantages of bioluminescence will help to illuminate answers as to why these complex genes have evolved.

3.3. QUORUM SENSING

Light production in many members of the Vibrionaceae is controlled by a phenomenon termed quorum sensing, or autoinduction. This density-dependent control of transcription is a unique form of bacterial communication using extracellular signaling molecules known as autoinducers (*N*-acyl-L-homoserine lactones; Dunlap 1997). As the number of cells in a population grows, autoinducers diffuse from the cell into the local environment. Once a threshold autoinducer concentration is reached, the autoinducer diffuses back into the cell, activating transcription.

Quorum sensing was first described in *V. fischeri* and has since been found to control a variety of different activities in a diverse number of microorganisms (see Dunlap 1997 for a complete review). In *V. fischeri*, the autoinducer created by LuxI interacts with the regulator protein (LuxR) to activate transcription of the *luxICDEABEG* operon, resulting in light production (Engebrecht and Silverman 1984). A second autoinduction system controlling luminescence in *V. fischeri* has also been identified (Gilson *et al*. 1995). This autoinducer, whose production is directed by *ainS* appears to interfere with the binding of the LuxI produced autoinducer to LuxR, effectively inhibiting luminescence at low densities of bacteria (Kuo *et al*. 1996). A transcriptional activator, LitR also appears to play a role in quorum sensing through regulation of *luxR* (Fidopiastis *et al*. 2002).

V. harveyi has proven to be a valuable organism for the comparative study of autoinduction of the *lux* genes. The complex *V. harveyi* regulation system lacks genes

homologous to *luxR* and *I* and autoinduction is controlled by a two component phosphorelay circuit (Bassler 1999). Briefly, the two autoinducers AI-1 and AI-2 interact with LuxQ and LuxN, respectively. The AI-1/LuxQ and AI-2/LuxN interaction is relayed to LuxO via the phosphotransferase, LuxU. LuxO indirectly inhibits transcription of the *luxCDABEGH* operon through a yet undiscovered repressor protein (Lilley and Bassler 2000).

Recent experiments have shown that virulence gene expression in *V. cholerae* is controlled by quorum sensing. Although *V. cholerae* lacks *luxCDABE* (genes for light production), genes analogous to those required for response to AI-2 in *V. harveyi* are present. In this system, LuxO inhibits transcription of *hapR*, which in turn inhibits *tcpP* transcription, an essential requirement for the expression of the toxin-coregulated pilus (TCP) virulence factor (Zhu *et al*. 2002).

4. Pathogenesis in the Vibrionaceae

4.1. DISEASES IN THE VIBRIONACEAE

Most diseases caused by vibrios are usually wound infections that have been in contact with saltwater or shellfish. These infections usually lead to "vibriosis" a terminal hemorrhagic septicemia in marine and freshwater fishes (Thune *et al*. 1993; Okuda *et al*. 2001), as well as in humans (Stelma *et al*.. 1992). Other *Vibrio* species, like *V. cholerae*, have been extensively studied due to their broad range of host virulence (Mooi and Bik 1997). Evolution of virulent strains has been of importance to human health and control of epidemics; although only a few serotypes have been fully characterized, there are still continual outbreaks of disease due to newly formed serotypes that are closely related to well studied strains. One example is that of the O1 serotype of *V. cholerae*; the main subdivisions of this strain are the classical and El Tor biotypes. These strains were linked to several outbreaks between 1881 and 1961, and yet a third serotype (O139 or Bengal) was discovered to be the culprit of a subsequent outbreak in India in 1992 (Mooi and Bik 1997). DNA fingerprinting confirms that the O139 strain is similar to El Tor and classic strains, however there are major differences that exist in several virulence factors that cause differences in virulence phenotypes. Thus, although strains are closely related phylogenetically, other factors have changed the mechanism as to which serotype invades or causes disease in its host.

Although *V. cholerae* is the most well-studied pathogenic *Vibrio* among the Vibrionaceae, other species of *Vibrio* have also been shown to contain similar virulence pathways that provide a means for infection and pathogenicity. Species such as *Vibrio (Listonella) anguilarum, V. parahaemolyticus, V. vulnificus*, and *V. mimicus* have been shown to express virulence-related properties such as production of the *toxR* gene (which regulates expression of the toxin-coregulated pilus (TCP) and cholera toxin; Lin *et al*. 1993; Okuda *et al*. 2001), production of phenolate siderophore (for iron sequestration; Stelma *et al*. 1992), as well as cell-mediated agglutination and bacterial adherence (Alam *et al*. 1996). Other benign mutualistic species, such as *V. fischeri*, have been shown to possess homologs to the transmembrane transcriptional activator *toxR* (Reich and Schoolnik 1994), and 2 types of halovibrin, which are both ADP-ribosyltransferases that have similar enzymatic activities to cholera toxin (Reich and

Schoolnik 1996; Reich *et al*. 1997). Since the infection mechanism is similar to *V. cholerae*, it is not surprising that *V. fischeri* possesses genes that have similar roles for infection and colonization of their respective eukaryotic partners (McFall-Ngai 2002; Nishiguchi 2002). Thus, proteins like cholera toxin and halovibrin are the "communication signals" that initiate interactions for bacteria to recognize and colonize their specific hosts.

4.2. HORIZONTAL GENE TRANSFER OF VIRULENCE FACTORS

As stated previously, genomic data has revealed that there is no strict congruence between phylogenetic relatedness and the physiological mechanisms that cause virulence. Several genes or gene families have been found that are responsible for transfer; cell-wall polysaccharide genes of several *V. cholerae* strains (El Tor, O139, O69, and O141) are associated with a mobile element (IS1358) that allows homologous recombination between lipopolysaccharide (LPS) gene clusters between different strains (Mooi and Bik 1997; Stroeher *et al*. 1995) Homologous gene clusters are also found in a number of other bacteria (not all pathogens), and may also play a role in mobilizing DNA directly by transposition to a phage (or plasmid) as well as in recombination. Both clinical and environmental isolates of *V. cholerae* have been found to contain the CTX phage, which encode the genes for cholera toxin (CT; Dalsgaard *et al*. 2001).

CTX is a lysogenic filamentous bacteriophage, and various isolates have been found in different strains of *V. cholera* (Davis *et al*. 1999). Analyses of these phage types have shown that some similarity is shared between phage repressor genes (*rstR*), which allow the phages to become infective or not (Davis *et al*. 1999). These phage are also known to use toxin co-regulated pili (TCP) as its receptor, and it has been hypothesized that this operon has also been introduced into strains of *V. cholerae* and related species through a bacteriophage (Dalsgaard *et al*. 2001; Mooi and Bik 1997; Nakayama *et al*. 1999; Nandi *et al*. 2000). Similarly, there are other virulence related genes that are clustered around the TCP operon as well as a putative integrase and bacteriophage attachment site (Mooi and Bik 1997). Thus, most of the current evidence points to the evolution of virulence through horizontal gene transfer via bacteriophage.

Although CTX demonstrates the acquisition of pathogenicity islands among virulent strains of *V. cholerae*, mutational studies have demonstrated that TCP production and regulation is not entirely responsible for colonization and adhesion to the host epithelia. The *rfb* genes in *V. cholerae* are responsible for the production of enzymes necessary for lipopolysaccharide biosynthesis, which was originally thought to be linked to the expression and assembly of TCP (Iredell and Manning 1997). Recent studies have shown that mutations in the *rfb* gene do not affect TCP production, but do cause inhibition of intestinal colonization (Chiang and Mekalanos 1999). Because this mutant did not affect TCP production, changes in bacterial LPS may cause more susceptibility to host immune responses (such as complement), or antibiotics. Several recent studies have shown that LPS from symbiotic strains cause host responsiveness and virulence (Foster *et al*. 2000; Zhang *et al*. 1997), and therefore play an important role along with TCP production in the infection of host tissues.

5. Conclusions

The family Vibrionaceae is one of the most diverse and widely distributed groups of prokaryotes that have radiated into hundreds of existing niches in the environment. Our present understanding of the evolution, ecology, and virulence of this important group of Proteobacteria has been greatly enhanced in the past decade. With future work on a number of specific *Vibrio* species in their free-living and symbiotic state, we can hope to uncover mechanisms of virulence, pathogenesis, and speciation within this dynamic family of microorganisms.

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