ABSTRACT

Title of Document: THE GENOME OF *VIBRIO ORIENTALIS:* A LUMINOUS BACTERIUM

Jinna Catherine Choi, Master of Science, 2012

Directed By: Dr. Rita R. Colwell, Distinguished Professor
Marine Estuarine Environmental Sciences
University of Maryland, College Park

Vibrio orientalis was first isolated from the Yellow Sea in China and described as a luminous bacterium. Since the bacterium was named, a surprisingly sparse amount of information is available. In this study, the genome of *V. orientalis* was sequenced; the draft genome consists of five contigs. The genome was explored using a comparative genomics approach to describe the genes that are in the genome. Genes and mobile elements were compared to other *Vibrio* species to determine the presence of mobile elements related to important cell functions and adaptive functions that provided evidence related to the environments in which the bacterium is able to adapt and survive. The genome also provided insight into nutrients that the bacterium may be able to metabolize.

THE GENOME OF *VIBRIO ORIENTALIS:* A LUMINOUS BACTERIUM

By

Jinna Catherine Choi

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2012

Advisory Committee:
Professor Rita R. Colwell, Chair
Professor Anwar Huq
Dr. Christopher J. Grim

Acknowledgements

I would like to express my appreciation and gratitude to my advisor, Dr. Rita R. Colwell, for her guidance and support of my research interests and encouragement to pursue my interests throughout my time in the program.

I would also like to thank Dr. Anwar Huq for his helpful advice on projects and communicating work, how to look at the bigger picture, and support in the laboratory.

I would like to thank Dr. Christopher Grim, who was a constant source of helpful advice in my journey to complete my thesis, encouragement to apply for the program, and guidance in applying my skills to projects.

I would like to thank Dr. Elisa Taviani, Bradd Haley, and Nur Hasan who helped me navigate through the laboratory, and were a sounding board for my ideas.

I would like to thank my family for their love and constant understanding.

Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

1.1 *Vibrio* genus

The genus *Vibrio* is a member of the *Vibrionaceae* of the γ-proteobacteria. Currently, the genus comprises over sixty species [1]. According to Bergey's Manual of Bacteriology, *Vibrio* species are Gram negative, motile rods, possessing at least one polar flagellum, are capable of facultative fermentation metabolism and are ubiquitous to aquatic environs [1, 2]. Vibrios are abundant in marine environments, having the ability to grow in a wide range of salinities and habitats, from fresh water to the deep sea [1, 3, 4]. Vibrios are important for aquaculture, and are involved in nutrient cycling, they possess the ability to degrade chitin and similar complex polymers and are found in high densities in association with marine organisms [1, 3, 4]. Relationships of *Vibrio* spp. with marine animals range from symbiotic to pathogenic and include fish, shrimp, crustaceans, and molluscs [1, 5-7]. Several species, mainly *V. cholerae, V. parahaemolyticus* and *V. vulnificus* are the cause disease in humans, gastrointestinal infections occur via consumption of contaminated food, usually seafood; and vibrios can also be associated with wound infections [1, 5]. *Vibrio* spp. are easily isolated from both environmental and clinical samples using selective and non-selective bacteriological culture plates [1, 8]. *Vibrio* spp. are able to grow in neutral and alkaline pH conditions and NaCl concentrations of 3% or higher can be used to select for *Vibrio* species [9].

1.2 *Vibrio* Genomes and Features

The genomes of *Vibrio* spp. contain two chromosomes [8]. This genome feature was first discovered by performing Pulse Field Gel Electrophoresis (PFGE) on *V. cholerae* and *V. parahaemolyticus*, concluding the presence of two replicons [10, 11]. Heidelberg et al. [12] published the first sequenced genome of the *Vibrio* genus, describing the genome of *V. cholerae* O1 El Tor N16961. The study confirmed the genome contains two asymmetrical chromosomes and revealed differences in gene content of each chromosome [12]. The large chromosome contained genes essential for growth, cell function, and pathogenicity [12]. The small chromosome possessed a majority of hypothetical protein-coding genes, genes that appear to have been acquired by horizontal gene transfer, and some essential genes [5, 12]. The small chromosome was described to also possess an integron island or super-integron, described as a capture system, whose genes are typically found on plasmids [12]. The authors concluded that this genome may be used to elucidate environmental characteristics and gene expression patterns important for survival in the natural environment and pathogenicity [12].

Okada et al. [8] assayed 34 *Vibrio* species and closely related species to determine their genome configuration. Genome configuration was elucidated through PFGE assays performed on undigested DNA. The study confirmed that the asymmetrical two chromosome feature is characteristic of all *Vibrio* species tested and is also characteristic of closely related genera, *Photobacterium* and *Salinvibrio*, suggesting stability of the two chromosome configuration in *Vibrionaceae,* compared with other bacterial species with multiple chromosomes [8]. The authors concluded that, generally, the large chromosome has a narrow size range (3.0 to 3.3. Mb), and the small chromosome has a more variable

size range (0.8 to 2.4 Mb) [8]. Studies have attempted to elucidate the different genes and functions of the two chromosomes in *Vibrio* species [5, 8]. Generally, the large chromosome contains essential genes that are needed for growth and viability, while the small chromosome contains genes that are needed for adaptation, including growth in different environments, but also contain genes that are essential for growth and survival, which may be a reason for its preservation in *Vibrio* spp. [5, 8, 13]. Possession of two chromosomes has been suggested to be retained because of its contribution to the ability of the *Vibrio* species to utilize adaptation genes to enhance survival [8].

1.3 Comparative Genomics

As the number of sequenced genomes increases at a fast rate, the ability to compare genomes to elucidate unique and shared characteristics has become more feasible. Whole genome comparisons can be advantageous to detect genomic element arrangements of a species compared with others at the group, species, and strain level. Comparative genomics can be used to determine relationships among closely related strains, providing new insight into the knowledge of the organism, survival, and pathogenicity not revealed through biochemical characterization [14]. Chun et al. [14] performed whole genome comparisons of twenty three *V. cholerae* strains by aligning genomes to infer the evolution of strains of *V. cholerae* through comparing genome organization and features. The study focused on the conversions of serogroups, the difference between the seventh pandemic and classical strains and identification and comparison of genomic islands present on each strain [14]. Comparisons on a smaller scale, such as sequences of genes or genomic elements can be used to determine relationships between features. For example, comparative genomics was used to assess

the genetic diversity of strains of *V. vulnificus* through comparing the variable number tandem repeat (VNTR) regions in different strains of the bacterium, utilizing diversity in one VNTR region that was used to subtype strains [15] Genes that are unique to pathogenic strains and species can be used as targets for identification and detection of species and virulent strains present in samples [16, 17]. For example, a multiplex PCR was developed to detect pathogenic strains of *V. vulnificus* but not other *Vibrio* spp, and non-pathogenic strains [17].

Species and strain classification can be altered on the basis of comparative genome analysis. For example, Haley et al. [18] identified two novel species of vibrios originally classified as *V. cholerae* through comparing genomes of the two species and found closely related species. Through comparing differences in average nucleotide identity, gene composition, arrangement, presence of genomic islands and prophages, and other genome features, the putative novel species were identified [18]. Next, Lin et al. [19] provided evidence of misidentification of two strains originally classified as *V. harveyi* utilizing comparative genomic hybridization and multi-locus sequence analysis. The two strains were identified as *V. campbellii* strains[19].

1.4 Evolution of *Vibrio* species

Vibrio species like many species evolve via genetic modifications, including mutations, gene duplication, lateral transfer of genes, and rearrangements that drive evolution and diversification of organisms [1, 13]. Mutations and sequence differences can be used to infer the approximate amount of divergence of two species [20]. Genomes can be compared to elucidate relationships of bacterial species based on the amount of sequence diversity of genes that are highly conserved, such as rRNA and housekeeping

genes [20]. The 16S rRNA and other housekeeping genes are used to construct phylogenies of species to infer evolution and relationships about species [1]. Gene duplication may result in functional divergence, which may enhance the ability to survive.

Horizontal or lateral gene transfer (HGT) drives evolution through incorporation of genetic material from one organism to another. The number of genes transferred and the ability of the species to be able to utilize foreign genes may offer new capabilities to the species [21]. For example, through HGT *Vibrio* species can become virulent [13, 22, 23]. Integrons and cassette systems are portions of genomes which have an enhanced ability to accept genes through HGT[21]. Vectors that carry genes from one genome to another include bacteriophages, transposons, and plasmids. Genomic Islands are clusters of genes that are acquired by HGT. HGT-introduced DNA sequences in genomes are detected through several methods to detect differences in genome sequences [22]. First, G+C% is used to identify regions that are not native to the genome. Next, codon usage of the acquired genes may also be different between native and newly acquired genes. The two methods are used to detect recently acquired genes [22]. Another method of detecting possible HGT is to compare phylogeny of a particular gene or gene cluster to organism phylogenies; phylogenies will be different if the genes of interest in the genome are a result of HGT [21, 22].

1.4.1 Integrons

Integrons are genetic systems that enable acquisition, rearrangement and expression of genes in gene cassettes through specialized regions in the genome where recombination can be easily achieved [24]. Integrons incorporate open reading frames

(ORF) into the genome through recombination and convert functional ability of the gene [25]. The essential components of an integron typically include an integrase family gene (*intI*), recombination sites, a primary recombination site (*attI*), and secondary recombination sites (*attC*) [26]. Superintegrons are a class of integrons present in genomes of γ-proteobacteria and other bacterial groups [5]. A majority of superintegrons are hypothesized to be unique to the host species and a majority of the ORF's have no known function, but contain some proteins involved in adaptive functions, such as antibiotic resistance [25, 27]. The superintegron of *Vibrio* spp. are large gene clusters ranging from 72 genes in *V. parahaemolyticus* to 200 in *V. vulnificus* [22]. Rowe-Magnus et al. [27] performed analysis on several superintegrons of *Vibrio* spp. and found that these genomic structures are at least 100 kb long, and most genes encode hypothetical proteins. Integron systems are advantageous because the ability to acquire and utilize foreign gene cassettes may enhance flexibility and fitness of organisms through rapid adaptation in changing environmental conditions [27] . The superintegron is found on the small chromosome in *V. cholerae*, and on the large chromosome in *V. parahaemolyticus* and *V. vulnificus* [5] The integron can be identified through a single gene recombination site [24].

1.4.2 Phages

Bacteriophages, or simply phages, are viruses that infect bacterial species, and have been found to be abundant in marine environments [5]. *Vibrio* spp. are associated with a wide variety of bacteriophages, most of which are harmless to the host, not related to virulence, and are not well characterized [28]. Phages are characterized into two types, lytic and lysogenic. Lysogenic phages integrate into the host DNA and multiply with the

host, the integrated form is termed a prophage. These phages are important for horizontal gene exchange, and are transferred via transduction, or lysogenic conversions [29]. In *Vibrio* species, phages can play an important role in pathogenicity, such as the CTXΦ phage in *V. cholerae* and *V. mimicus,* the f237 phage in *V. parahaemolyticus,* and VHML in *V. harveyi* [1, 13, 23, 30, 31]. CTXΦ prophage is a lysogenic phage that codes for the cholera enterotoxin, the principal virulence factor of toxigenic *V. cholerae* and *V. mimicus,* and other virulence factors essential for infection of the human host [5, 23, 32]. The f237 phage is associated with several serovars of *V. parahaemolyticus,* including O3:K6 and other emerging serovars that are related to pandemic spread of disease caused by *V. parahaemolyticus* [30]. Genomic analysis can be used for both identification and sequence analysis of phages [5].

1.4.3 Genomic Islands

Genomic Islands are large DNA clusters that have been acquired by HGT. Genomic islands are linked to pathogenicity potential in several *Vibrio* species. In *V. cholerae,* pathogenicity island 1 and 2 (VPI-1 and VPI-2) are present in all *V. cholerae* O1 and O139 serotypes that are toxigenic and VSP-1 and VSP-2 are specific to the seventh pandemic strains of *V. cholerae*. These islands carry genes that enhance the virulence potential of pathogenic *V. cholerae* strains [14, 33, 34]. In *V. parahaemolyticus*, genomic islands V-Pal-1, 4, 5, and 6 are present on all pandemic strains [35] . In *V. vulnificus*, differences in genomic islands in strains have been suggested to be a source of genome plasticity and a genomic island, RegionXII, has been linked with higher pathogenic potential of virulent strains [36, 37]. Genomic islands can be identified through comparative genomics, searching for common characteristics of

genomic islands, including differences in codon usage compared with the rest of the genome, the G+C% content and genes that are related to mobile elements [38, 39].

1.5 Vibrios and the Environment

Vibrio species are ubiquitous to the marine environment. Vibrios are widely distributed around the world and have been isolated from a variety of environments, ranging from coastal, open and deep water environments [1, 40-43]. *Vibrio* species can constitute up to 40% of the total bacterial populations in coastal and ocean environments [3]. Little is known about the distribution and ecology of *Vibrio* species in benthic environments, however it has been suggested that it serves as a reservoir for *Vibrio* species, including *V. parahaemolyticus* and *V. cholerae* [3, 40]. In one study, the population of *Vibrio* species in the benthic environments did not change, while the water column was found to be dynamic [40].

1.5.1 Temperature, Salinity and Growth of *Vibrio* species

Temperature significantly influences abundance of *Vibrio* species and exerts a domint impact on isolation and growth of these organisms. Temperature accounts for about 48% of variability of isolation of *Vibrio* species, including 47% in *V. vulnificus* according to one report [44]. In general, *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* show a pattern of higher abundance and more frequent isolation in summer months, directly correlated with changing temperature [40, 44, 45]. Salinity also associated with the isolation and growth of *Vibrio* species and the latter generally are inversely correlated with salinity, however, population dynamics of each species is different based on their optimal salt requirement, for example, between 5 to 10 ppt for *V. vulnificus* and 2 to 14 ppt for *V. cholerae* [45, 46].

1.5.2 Vibrios and association with aquatic organisms

Commensal association with aquatic organisms is considered a survival strategy of vibrios in the marine environment, providing habitat, vehicles of transport, and protection from predators [7]. These organisms are commonly found in association with marine animals, such as chitinous organisms, plankton, and algal cells [3, 7]. *Vibrio* spp. are capable of utilizing chitin, a polymer of N-acetylglucosamine (GlcNAc), one of the most abundant polymers in nature and have been suggested to contribute to the breakdown of other polymers in the marine environment [3, 47]. *Vibrio* species associate and attach to chitinous organisms, such as the exoskeletons of zooplankton, that provide habitat and nutrients for several *Vibrio* species, including *V. parahaemolyticus, V. cholerae, V. vulnificus, V. mimicus,* and *V. cincinnatiensis.* These associations represent a notable portion of *Vibrio* species in the natural environment [48-50]. Zooplankton associations can account for up to 40% of *Vibrio* species in the water column in the Chesapeake Bay and vary with respect to species and season [41]. *Vibrio* species are associated with large and small zooplankton and these associations may offer a competitive advantage over other heterotrophic bacteria, which are not able to attach to zooplankton [48-50]. Attachment to zooplankton is suggested to help survival of *Vibrio* species in winter months, by providing protection from colder temperatures [49]. Zooplankton blooms are correlated with *Vibrio* populations, having the same seasonal abundance patterns, with the highest numbers in the spring and summer months [48, 50]. Vibrio species also participate in symbiotic relationships. The association of *V. fischeri* with *Euprymna scolopes* is mutually beneficial, where *V. fischeri* provides luminescence to the squid species for protection against predators, and *V. fischeri* colonizes the light organ of the squid species [6, 51-53].

1.5.3 Vibrios and nutrients

Vibrio spp. are involved in the cycling of important nutrients, including carbon, nitrogen and phosphorus in the marine environment [4]. These organisms can adapt to grow in both high nutrient and nutrient-limiting environments utilizing organic matter as a carbon source [4]. The ability of *Vibrio* spp. to store excess carbon may provide an advantage in nutrient limited environments and competition for nutrients. In carbon limited environments, *V. cholerae* alters expression and may enhance virulence [4, 54]. *Vibrio* species are involved in nitrogen fixation, nitrate reduction, and ammonification [4]. Gene targets for *nifH* and *nasA* can be used to detect the ability of nitrogen fixation and nitrate reduction respectively. The widespread presence of the *nasA* gene in *Vibrio* spp. suggests that vibrios are able to consume nitrate and reduce nitrate to ammonia [4, 55] . Chitinases and proteases hydrolyze nitrogenous polymers to simpler units and recycle them into the environment, producing one of the largest pools of amino sugars in the oceans [4, 56]. Phosphorous is utilized by *Vibrio* species, for functional biological processes, including nucleic acid and phospholipid synthesis. Alkaline phosphatase works to supply phosphate pools when phosphorus is limited and has been detected in several *Vibrio* species [4]. 5' Nucleotidase is an enzyme that supplies phosphorus, and activity is not limited by high levels of phosphorus. It has been detected in all *Vibrio* genomes and works to increase phosphate pools [4]. Vibrios are also able to break down toxic polycyclic carbons, found in marine sediments [1]

1.5.4 Environmental Stressors

Vibrio species have evolved to be able to survive in response to environmental stressors. Environmental stressors include changing temperature, salinity, pH, grazing, nutrient limitation, and combinations of these factors. *Vibrio* species in nature have rapid growth cycles when nutrients and growth conditions are favorable and have long generation times and non-growth periods when conditions are not ideal for growth [57]. Presence of genes related to the ability to respond to changes in the environment in the genome may give insight into the adaptation abilities of a species to survive when facing changing environmental conditions. *Vibrio* species populations can change throughout the year, and water temperature is a good predictor of the occurrence of different species [58]. Temperature shock genes are present in many *Vibrio* species and aid the ability of species to adapt to changing temperatures [59, 60]. For example, exposure to cold temperatures resulted in up-regulation of two cold shock proteins in *V. parahaemolyticus*, and expression of one protein was correlated with decreasing temperatures [59]. The expression of the other protein was initiated and stayed at a constant expression level during exposure to cold temperatures [59]. Adaptation to changes in pH is also facilitated by specific genes and operons, specifically for response to acidic conditions [61, 62]. The ability to form biofilms aid the survival of *Vibrio* species by protecting against grazing pressures, UV light, acidic conditions, dehydration, oxidative environments and antimicrobial agents.[63]. Biofilm formation is dependent on the ability to produce exopolysaccharides and is associated with the rugose morphology of a cell, quorum sensing, and *hapR* [57].

1.5.5 Quorum Sensing

Quorum sensing is a process where cells detect cell density via extracellular signaling molecules called autoinducers, which increases as cell density increases [64-66]. Accumulation leads to activation of signaling cascades that lead to coordinated cellular processes[64-66]. Quorum sensing coordinates cellular processes that are beneficial with high cell density, including bioluminescence, virulence gene expression, and biofilm formation [66, 67]. In *V. harveyi* and *V. cholerae,* parallel sensing systems exist that respond to different quorum molecules and activate appropriate signaling cascades in response to the density of different cells [65, 67]. Autoinducer (AI)-2, first described in *V. harveyi,* is subsequently found in a wide range of bacteria, and the signaling cascade that responds to AI-2 is suggested to be identical in all species [66, 68, 69]. AI-2 production is dependent on the presence of the gene *luxS* and *luxS* mutants lack AI-2 production [66, 69]. AI-2 has been suggested to be able to regulate interspecies cell-to-cell communication [66, 69]. The AI-2 in *V. harveyi* controls expression of the density dependent luciferase operon [66, 69]. When AI-2 accumulates, it causes activation of a gene cascade linked with de-activation of a repressor, in turn linked with transcription of a number of processes in *V. harveyi* and *V. cholerae* [65, 67].

1.6 *Vibrio* infections

Several *Vibrio* species are characterized as the causative agent of disease in marine animals and humans. Fish pathogens include *V. anguillarum, V. damsela, V. parahaemolyticus, V. vulnificus, V. harveyi,* and *V. alginolyticus* [1]. *V. anguillarum* affects a variety of fish species, including salmon and rainbow trout, serotypes O1 and O2 are associated with fish infection [70]. *V. damsela* has been isolated from ulcers in

damselfish and human wounds [71, 72]. *V. harveyi* is a serious pathogen and is able to infect a range of animals, such as shrimp and sharks; however, mechanisms for infection have yet to be fully understood [73]. It has been suggested that virulence is acquired through mobile elements because of the difference in phylogenies between species and virulence factors of several *Vibrio* species [73]. Corals are infected by *V. choralliiltyicus* and *V. shilonii,* that have been linked to coral bleaching [1].

Vibrio species infect humans upon consumption of contaminated water and food, and are also associated with systemic and wound infections [1, 44, 74]. Eleven *Vibrio* spp. have been linked to human infection, however, three species, *V. cholerae, V. vulnificus,* and *V. parahaemolyticus* are very important because of the diseases they cause are globally distributed, and has devastating effect [5, 75]. There are more than 250 serogroups of *V. cholerae*, of which only serogroups O1 and O139 are capable of causing epidemics. They contain multiple virulence factors and produce cholera toxin and toxin co-regulated pilus, essential for infection [5, 75, 76]. *V. parahaemolyticus* causes over half of all food poisoning outbreaks of bacterial origin and has a wide range of serovars that are linked with disease. However, the O3:K6 serovar has emerged to be the main disease-causing serovar in recent years [5, 75, 77]. The chief virulence factor of pathogenic *V. parahaemolyticus* is its thermostable direct hemolysin (TDH) [5, 75, 78]. *V. vulnificus* has a large heterogeneity in the infections it causes, where three biotypes are linked with disease and biotype 1 is predominantly associated with human disease [5, 79]. *V. vulnificus* has been described as an opportunistic pathogen, and its major virulence factor is the capsular polysaccharide (CPS) and iron availability of the host [75,

79]. Host susceptibility also plays a large role in the progression of disease in *V. vulnificus* [80].

1.7 Luminescence

Vibrio spp. are among the most widely studied bacteria with respect to bioluminescence [81]. Bioluminescence is catalyzed by the luciferase enzyme in the presence of oxygen. Luminescent bacteria are found in the ocean, free living or often in symbiotic relationships [81]. For example, the bacterium receives nutrients and a habitat optimal for its growth and the host receives ability of bioluminescence to protect it from predators, like the relationship of *V. fischeri* with *Euprymna scolopes* and in *V. logei* and two squid species [6, 51-53, 82]. Luminescence is dependent on density of the cells, and luminescence related transcription is under the control of a repressor, and transcription is activated at high cell densities when it is beneficial [65, 69, 81, 83].

1.7.1 *lux* Operon

All bioluminescent strains of *Vibrio* species possess the lux operon, which is linked with the ability of bioluminescence [81]. The core of the *lux* operon is the gene cluster of five genes, *luxCDABE* associated with all *lux* systems in luminescent bacteria [81]. The *luxA* and *luxB* genes code for the alpha and beta subunits of luciferase, forming heterodimer luciferase enzymes that catalyze luminescence. The two genes have 30% amino acid identity, suggesting they arose from gene duplication [81]. *luxC, lux D, and luxE* are the three genes that form a fatty acid reductase complex [84]. The *lux* operon is regulated by quorum sensing and in *V. harveyi* the *lux* operon transcription is controlled by the *luxO* gene [85]. *V. fischeri* is the only species that contain regulatory genes in association with the lux operon [84]. Urbanczyk et al. [86] concluded that the *lux* operon

is predominantly vertically transferred. They also suggested that there are rare HGT events responsible for *lux* operon acquisition, as is the case for *V. vulnificus* [86]. In this case, the likely donor is *V. harveyi,* due to the presence of the *luxH* gene in the operon, and the close relationship to *V. harveyi* in phylogenetic analysis [86]. *V. vulnificus* strain VVL1 is the only strain of *V. vulnificus* that is luminescent and the presence of the *lux* operon and luminescence capabilities suggests that this strain acquired these genes through HGT [86].

1.8 Vibrio orientalis

Vibrio orientalis was first isolated and described in 1983 with a group of luminous bacteria from the coast of China in the Yellow Sea [87]. The luminous isolates were characterized by assessing phenotypic characteristics, including swarming, shape of cells, flagella, and ability to utilize 82 organic compounds as energy sources [87]. The G+C% content of DNA (45.4) was determined by density gradient analysis [87]. *V. orientalis* was described as a novel species because of its ability to accumulate poly- β-hydroxybutyrate as a reserve product and utilize DL- β-hydroxybutyrate, putrescine, and sperimine as sole or principle sources of carbon, characteristic only to this species, compared with other luminescent bacteria isolated [87]. *V. orientalis* grows at 4°C, but not at 40°C [87]. It also utilizes sucrose and cellobiose as sole or principle sources of carbon, but not glucuronate, α-ketoglutarate, and γ-aminobutyrate [87]. Anti-serum to the iron-containing superoxide dismutase of several *Vibrio* species was used to determine that *V. orientalis* is a member of the genus *Vibrio* [87]. *V. orientalis* ATCC 33934 (CIP102891) is additionally characterized by having a single, sheathed polar flagellum, and is the type strain of the species [87].

After the initial characterization of *V. orientalis*, there have been only a sparse number of publications related to *V. orientalis*, with a majority of publications mentioning the bacterium in relation to luminescence genes and ability, as a reference strain in papers describing novel species, and in description of the taxonomy of the genus *Vibrio* [88, 89]. *V. orientalis* has also been isolated in the western Mediterranean Sea while in studies of *V. vulnificus*, along with several other *Vibrio* spp[90]. Interestingly, in this study, *V. orientalis* was not isolated at temperatures below 15°C [90].

In this study, the genome of *V. orientalis* CIP 102891 is described to further understand its growth and survival mechanisms, comparing them to other available *Vibrio* species genomes. This was carried out by employing a comparative genomics approach to describe genome characteristics and features.

The *V. orientalis* genome was explored to understand its relationship to its environment and ecological conditions. By exploring adaptation mechanisms and survival related genes, it may give some insight into what environs this bacterium may potentially be isolated. These may help increase the ability to isolate and test for the presence of this organism when identifying related species. The study also explored virulence related mechanisms in virulent *Vibrio* spp. found on the *V. orientalis* genome that may not necessarily indicative of virulence potential. The presence of these virulence related genes may be important for environmental function.

Chapter 2 Materials and Methods

2.1 Genome Sequencing

Draft sequences were obtained from a blend of Sanger and 454 sequencing technologies and involved paired end Sanger sequencing of 8kb plasmid libraries with 5x coverage data, 454 pyrosequencing with 20x coverage data, and optional paired end Sanger sequencing with 35kb fosmid libraries to 1-2X coverage data. The optional paired end sequencing was done based on complexity of the repeats. The combined data gave a total of 6.5x coverage. To construct the contigs, a collection of custom software and targeted reaction types were used. Phred/Phrap/Consed software package was used to assemble the reads for quality assessment. Solexa data was used to help distinguish low quality regions and to help with some gap closure. Missing-assemblies were corrected with Dupfinisher and with transposon bombs of the contigs. Gaps were closed using Consed, primer walking, or PCR reactions. Gene finding and annotation were achieved using an automated annotation serve, RAST [91].

2. 2 Comparative Genomics

Dispensable and unique genomic regions were found using whole genome comparisons with closely related species. Genome to genome comparisons were completed using approaches described by Chun et al. [14] to compare the genome of *V. orientalis* CIP102891 to available *Vibrio* genomes in the pipeline. The homologous regions were identified and used to assess relationships between the species. Nucleotide similarity was calculated through pairwise alignment of genes and gene clusters using the

Geneious interface [92]. Gene clusters and region compositions were discovered and compared using Rapid Annotations using Subsystems Technology(RAST) [91].

Average nucleotide Identity (ANI) was calculated using a reciprocal BLASTN analysis for the genome. The average similarity between genomes was measured for all conserved protein-coding genes, following the methods of Konstantinidis and Tiedje [93]. ANI was calculated using a java based interface, the Jspecies program [94]

2. 3 Identification of Genomic Islands

Putative genomic islands (VoGI) were identified on the draft genome using the web-based application program IslandViewer [95]. The program uses three methods to find genomic islands in sequences. It uses two sequence composition methods and one method for comparative analysis [95]. The genomic islands that were found were numbered VoGI1 through VoGI4. Each genomic island (GI) was compared to known sequences using NCBI BLASTX to search for regions of the islands that showed significant similarity to other species of the genus *Vibrio.* Regions were compared to other species with similar genes and order using RAST to compare contents of the island [91].

2. 4 Sequence Alignment

Multiple sequence alignment was achieved through ClustalW [96]. ClustalW alignment is based on how similar each sequence in the multiple alignments is to each other using pairwise comparisons of all of the sequences. Next, it takes into account amino acid substitution matrices, residue specific gap penalties, and locally reduced gap penalties, and gives more leniencies to positions at the beginning of the alignments. Multiple sequence alignments were constructed utilizing the MEGA5 program [97] .

2. 5 Phylogeny Construction

Multiple sequence alignments from MEGA5 were used to construct phylogenetic trees using neighbor-joining statistical methods in the MEGA5 program [98]. The distances were calculated using the Kimura-2 evolutionary model [99]. Bootstrap values were also constructed using the MEGA5 program.

Chapter 3 Results and Discussion

3.1 Genome Features of *V. orientalis*

The genome of *V. orientalis* CIP102891 (Accession number ACZV00000000) compromises ca. 4,698,244 base pairs (bp), with a G+C% content of 44%. The genome encodes 128 structural RNAs and 4,303 open reading frames (ORF), as predicted by RAST. Like all other *Vibrio* species, the *V. orientalis* genome contains two chromosomes. The large chromosome (C-I), not closed, consists of four contigs (ctg 76, 77, 78 and 80). The small chromosome (C-II) is closed (ctg 79) and is 1,579,959 bp.

The average nucleotide identities between *V. orientalis* and several *Vibrio* species are shown in Table 1. The ANI was calculated based on BLAST [94]. Based on the table below, the highest ANI is with *Vibrio harveyi*, *Vibrio* EX25 and *Vibrio parahaemolyticus,* which suggests a close relationship among these genomes.

Table 1. Average Nucleotide Identity of *V. orientalis* CIP102891 to other *Vibrio* species

Species	ANI
Vibrio anguillarum	72.69
Vibrio cholerae	71.93
Vibrio EJY3	74.06
Vibrio Ex25	74.7
Vibrio fischeri	71.37
Vibrio furnissii	72.23
Vibrio harveyi	74.93
Vibrio parahaemolyticus	74.14
Vibrio splendidus	73.72
Vibrio vulnificus	73.03

V. orientalis contains seven rRNA operons, compared with *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, which have eight, ten and eight operons, respectively. The *V. orientalis* genome contains more rRNA operons than the genomes of *V. alginolyticus, V. coralliilyticus,* and *V. shilonii,* and the same number as *V. mimicus.* The relative fitness of *V. orientalis* can be inferred by rRNA copy number. The number of rRNA copy numbers infers the relative ability to adapt to changing ecological conditions, response time, and growth rate in response to an influx of nutrients and resources that are beneficial [100]. As little as a loss of one rRNA copy number can result in the decreased ability to grow rapidly in response to exposure to beneficial conditions, and increased lag time to initiate growth, and decreased growth rate [100].

3.2 Housekeeping gene based phylogeny

The phylogeny of the vibrios was constructed using seventeen housekeeping genes listed in Table 2. All housekeeping genes were annotated in RAST and were downloaded off the RAST annotation website for consistency when constructing the trees [91]. Each housekeeping gene was separately aligned through multiple sequence analysis. The aligned sequences were concatenated to construct a tree and fifteen species were compared (Figure 1). Based on this phylogenetic tree, *V. orientalis* is most closely related to *V. coralliilyticus*, a coral pathogen, whose pathogenicity is dependent on temperature [1]. The phylogenetic analysis also confirms that *V. orientalis* is closely related to *V. parahaemolyticus, V. harveyi,* and *Vibrio* sp EX25. The tree has a different phylogeny than the phylogeny presented by Thompson et al. [1].

Table 2. Genes used to construct a phylogenetic tree.

Gene	Product	EC number
aminopeptidase P		EC 3.4.11.1
alaS	alanyl-tRNA synthetase	EC 6.1.1.7
aspS	aspartyl-tRNA synthetase	EC 6.1.1.12
gltX	glutamyl-tRNA synthetase	EC 6.1.1.17
gyrB	DNA gyrase subunit B	EC 5.99.1.3
hisS	histidyl-tRNA synthetase	EC 6.1.1.21
ileS	isoleucyl-tRNA synthetase	EC 6.1.1.5
infB	initiation factor 2	
metG	methionyl-tRNA synthetase	EC 6.1.1.10
mreB	rod shape-determining protein MreB	
pntA	transhydrogenase alpha subunit	EC 1.6.1.2
pheT	phenylalanyl-tRNA synthetase beta chain	EC 6.1.1.20
pyrH	uridylate kinase	EC 2.7.4.-
rpoB	RNA polymerase, beta subunit	EC 2.7.7.6
rpsH	30S ribosomal subunit protein S8	
topA	Topoisomerase I	EC 5.99.1.2
valS	valyl-tRNA synthetase	EC 6.1.1.9

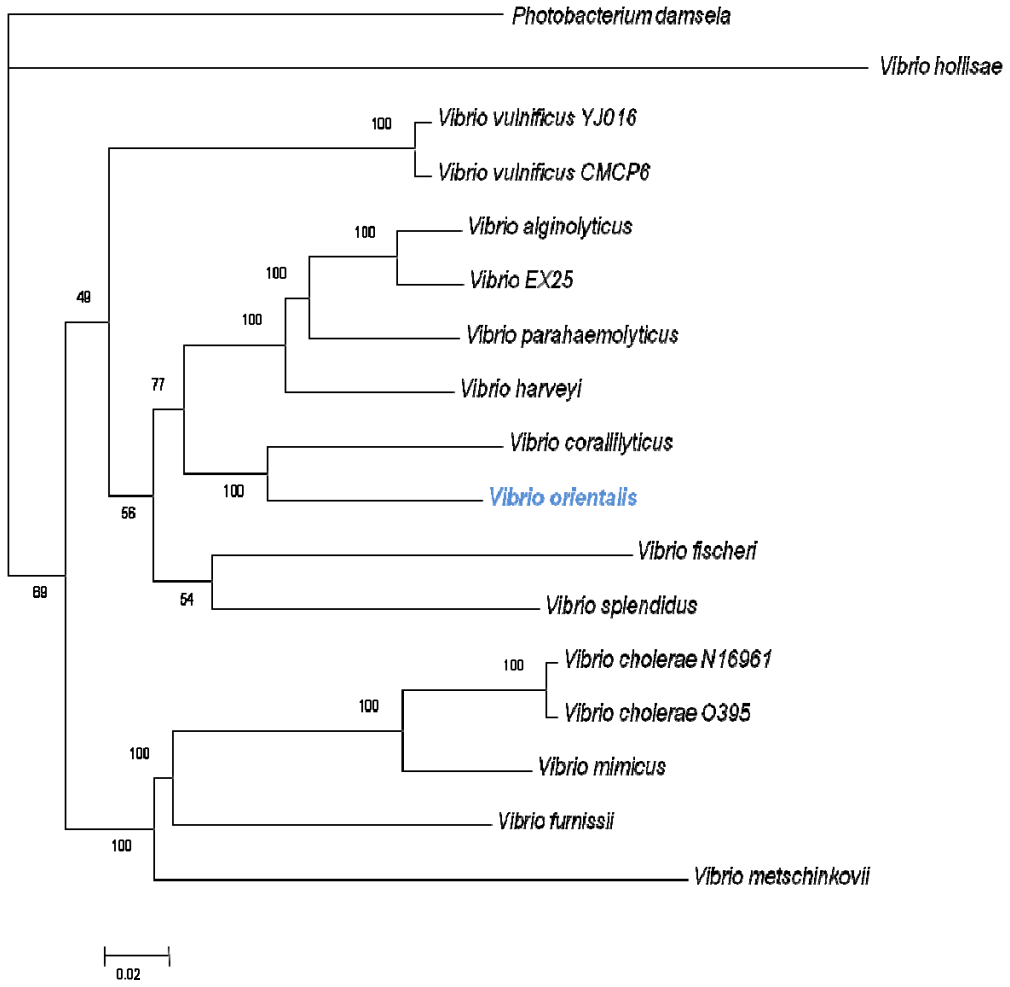

Figure 1. Phylogenetic tree based on seventeen housekeeping genes. This tree was constructed using the neighbor-joining statistical model and distances were calculated using the Kimura-2 parameter model. Bootstrap values are shown at the junctions. V. orientalis is highlighted in blue.

3.3 Mobile Elements

3.3.1 Genomic Islands

Four putative genomic islands were found using IslandViewer [95] and are named VoGI1 through VoGI4. VoGI1 and VoGI2 are found on ctg 78 (C-I). VoGI3 and VoGI4 are found on ctg 79(C-II). Fig. 2 shows the location of each genomic island on each contig. The figures are outputs of IslandViewer. On contig 78, the genomic islands are situated closely on the genome.

VoGI1 (VIA_00509 to VIA_00535) is 30,396 bp has a G+C% content of 39%. Genes related to O-antigen biosynthesis are found on the island. The O-antigen biosynthesis region in several *Vibrio* species is flanked by a *gmhD* (also *rfaD*) gene and the *rjg* gene [101, 102]. The *gmhD* gene (ADP-L-glycero-D-manno-heptose-6-epimerase) (VIA_00536) flanks the island in *V. orientalis* and is downstream of the *wblB* gene. This gene has 76% similarity with that of *V. cholerae* and 79% similarity with *V. parahaemolyticus.* The *rjg* gene is not present on the island, but is found on ctg80 (VIA_2224), and shows 69% similarity to *V. cholerae* MO45 *wbfZ* gene and 67% similarity to *V. splendidus* LPG32 beta-lactamase fold involved in RNA processing. Two genes in VoGI1show high similarity to the O-antigen synthesis genes of *V. cholerae* O22, the *wblB* gene and the UDP-glucose dehydrogenase encoding gene (VIA_000534 and VIA_000535). The *wblB* gene coding region is part of serogroup O22 biosynthesis and has been described as highly similar to *lacA* in *E. coli* [103]. The *wzm* gene is also found in the island and may possess a similar function to the *V. cholerae wzm* gene that codes an integral membrane protein acting as a pore that exports the O-antigen [102, 104]. The

genes that are present in VoGI1 are listed in Table 3. VoGI1 likely codes for the genes necessary for O-antigen synthesis of *V. orientalis.*

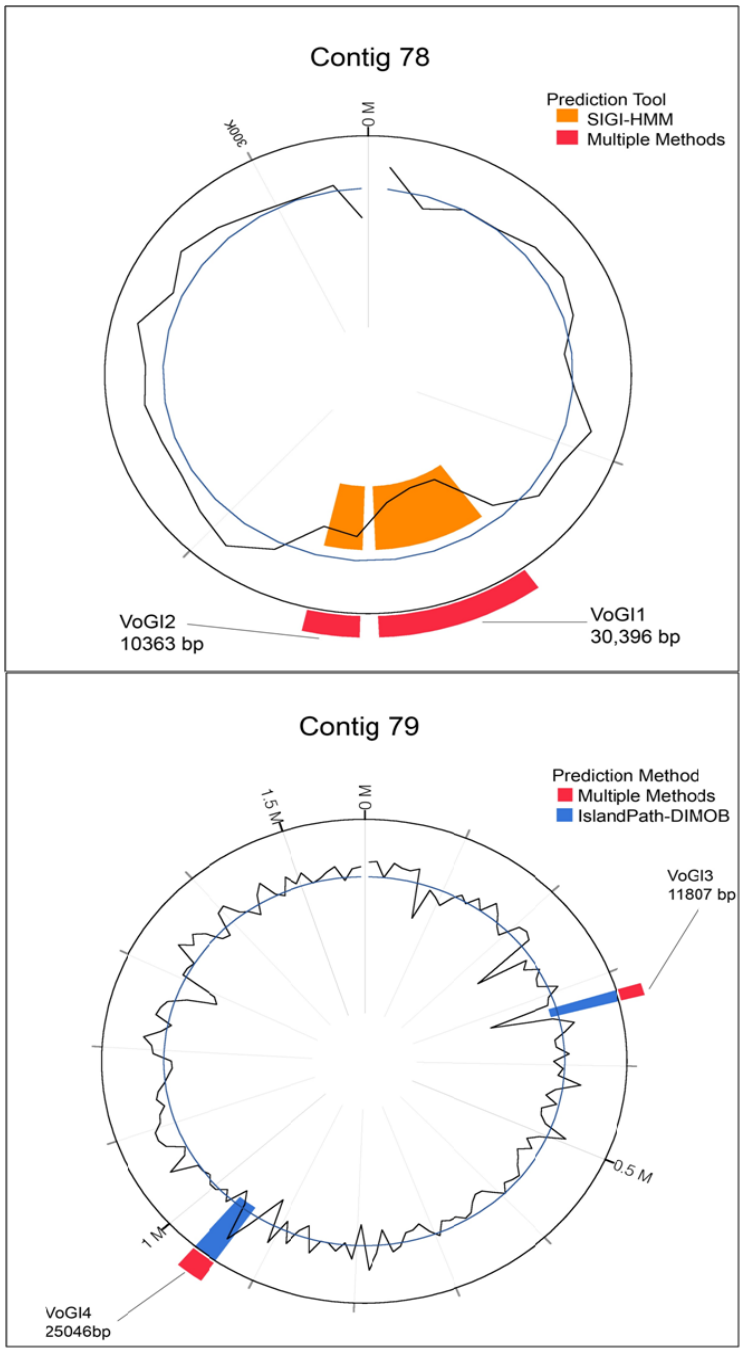

Figure 2. The outputs of IslandViewer show the location of each genomic island on the contig where it is found. The different colors represent the method used to predict the genomic islands on each contig shown in the legend.

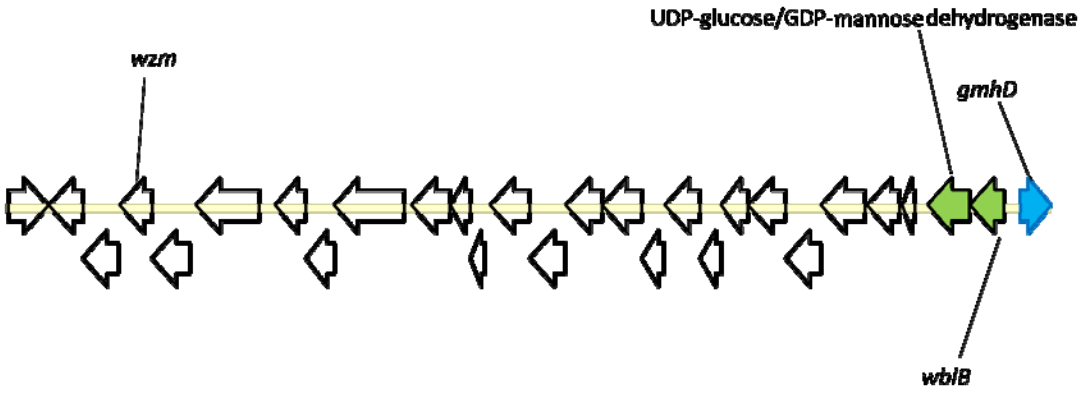

Figure 3. VoGI1 of *V. orientalis.* The flanking gene, *gmhD* is blue. Genes similar to *V. cholerae* O22 synthesis are green and the 26 ORF's present on the island are defined by black arrows.

Table 3. Genes and flanking genes of VoGI1.

Locus ID	Product
VIA_000508	CDP-diacylglycerol--serine O-phosphatidyltransferase (EC 2.7.8.8)
VIA_000509	UDP-glucose lipid carrier transferase
VIA_000510	COG0438: Glycosyltransferase
VIA_000511	glycosyltransferase
VIA_000512	Wzm
VIA_000513	hypothetical protein
VIA_000514	Nucleoside-diphosphate sugar epimerase/dehydratase
VIA_000515	putative glucosyl transferase
VIA_000516	hypothetical protein
VIA_000517	hypothetical protein
VIA_000518	pilin glycosylation protein
VIA_000519	pilin glycosylation protein
VIA_000520	Lipid carrier : UDP-N-acetylgalactosaminyltransferase (EC 2.4.1.-)
VIA_000521	probable glycosyltransferase
VIA_000522	putative glycosyl transferase
VIA_000523	putative glycosyl transferase
VIA_000524	hypothetical protein
VIA_000525	hypothetical protein
VIA_000526	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)
VIA_000527	hypothetical protein
VIA_000528	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase (EC 2.3.1.-)
VIA_000529	Glycosyltransferase
VIA_000530	hypothetical protein
VIA_000531	glycosyl transferase, group 1
VIA_000532	DegT/DnrJ/EryC1/StrS family protein
VIA_000533	probable acetyltransferase
VIA_000534	UDP-glucose/GDP-mannose dehydrogenase
VIA_000535	WblB protein
VIA_000536	ADP-L-glycero-D-manno-heptose-6-epimerase (EC 5.1.3.20)

VoGI2 (VIA_000539 to VIA_000550) is 10,363bp and has a G+C% content of 41%. A majority of the genes present on this island are linked with core oligosaccharide synthesis. In *V. cholerae,* the O-antigen synthesis gene cluster and the core oligosaccharide biosynthesis cluster are adjacent to one another and share the gene *gmhD* (VIA_000536) [102]. The *rfaD* gene has been documented to be required for lipopolysaccharide synthesis [105]. *Ca*. 75% of the island shows nucleotide similarity to *V. splendidus* LGP32 (VIA_00540 to VIA_00546) with overall similarity of 70%, as seen in Fig 4. Core oligosaccharide in *Vibrio* species is similar. Two genes, phosphopantetheine adenylyltransferase and ADP-heptose--lipooligosaccharide heptosyltransferase II, found on VoGI2 show at least 60% nucleotide similarity to several species in the *Vibrio* genus, including *V. cholerae, V. vulnificus, V. fischeri, V. alginolyticus and V. parahaemolyticus* and are linked to core lipopolysaccharide synthesis. The lipid A synthesis gene is present between VoGI1 and VoGI2 (VIA_0537). The genes on the island are listed in Table 4. VoGI2 likely encodes the genes required for biosynthesis of core oligosaccharide synthesis of *V. orientalis.*

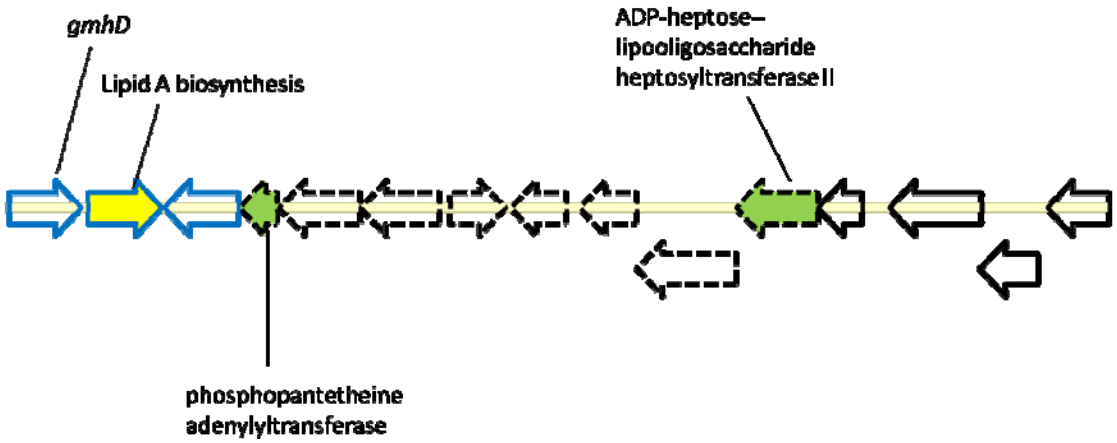

Figure 4. VoGI2 of *V. orientalis*. 12 ORF's that are a part of the island are outlined in black. The genes not calculated to be in the island are outlined in blue. Genes conserved in the *Vibrio* genus that have similarity are green. Lipid A biosynthesis gene is shown in yellow. ORF's that are similar to *V. splendidus* are outlined in a dashed pattern.

Table 4. VoGI2 genes and flanking genes

Locus ID	Product
VIA_000538	Glycosyltransferase
VIA_000539	Phosphopantetheine adenylyltransferase (EC 2.7.7.3)
VIA_000540	ADP-heptose--lipooligosaccharide heptosyltransferase II (EC 2.4.1.-)
VIA_000541	putative LPS core biosynthesis-related protein
VIA_000542	3-deoxy-D-manno-octulosonic acid kinase (EC 2.7.1.-)
VIA_000543	hypothetical protein
VIA_000544	3-deoxy-D-manno-octulosonic-acid transferase
VIA_000545	3-deoxy-D-manno-octulosonic-acid transferase (EC 2.-.-.-)
VIA_000546	ADP-heptose--lipooligosaccharide heptosyltransferase II (EC 2.4.1.-)
VIA_000547	putative lipopolysaccharide A protein
VIA_000548	O-antigen ligase
VIA_000549	3-deoxy-D-manno-octulosonic-acid transferase
VIA_000550	Formamidopyrimidine-DNA glycosylase (EC 3.2.2.23)

Table 5. VoGI3 and flanking genes

Locus ID	Product
VIA_000969	Peptidyl-prolyl cis-trans isomerase ppiC (EC 5.2.1.8)
VIA_000970	Recombinase
VIA_000971	hypothetical protein
VIA_000972	hypothetical protein
VIA_000973	hypothetical protein
VIA_000974	hypothetical protein
VIA_000975	hypothetical protein
VIA_000976	hypothetical protein
VIA_000977	type IIs modification methyltransferase
VIA_000978	restriction endonuclease
VIA_000979	hypothetical protein
VIA_000980	hypothetical protein
VIA_000981	hypothetical protein
VIA_000982	Cytochrome c4

VoGI-3 is found on contig 79 and is 11,807bp (VIA_000969 to VIA_000982), with G+C% of 37%. VoGI3 has no significant similarity to any known sequences in the NCBI database. The majority of the island consists of hypothetical proteins and is flanked by a peptidyl-prolyl cis-trans isomerase, and cytochrome c4. This island also includes recombinase gene, methyl-transferase, and restriction endonuclease.

VoGI-4 is found on contig 79 and is 25,046bp (VIA_001514 to VIA_001545) with G+C% content of 45%. VoGI-4 contains genes related to bacteriophage incorporation and synthesis, including a head protein, and a probable bacteriophage integrase. Bacteriophage related genes are flanked by a hypothetical protein (VIA_001505) and an integrase gene (VIA_001543) found on the island. These genes show high similarity to genes flanking bacteriophage K139. The nucleotide sequences of the hypothetical protein of *V. orientalis* and phage protein found on bacteriophage K139 are 63% similar, and the intergase genes found on the two genomes are 68% similar. The genes in the island have a similar gene arrangement to bacteriophage K139. Bacteriophage K139 is found in *V. cholerae* O139, characterized as a lysogenic prophage and is also present in all *V. cholerae* O1 classical strains [106]. The order of genes is almost identical between VoGI-4 and *V. cholerae* O139. The phage replication protein gene is similar to *V. cholerae, V. parahaemolyticus, V. shilonii,* and *V. harveyi*. The relative organization of the genes is also similar to *V. harveyi* VHML phage [107]. The presence of this island suggests that *V. orientalis* incorporated a bacteriophage and codes for a prophage incorporated into the genome.

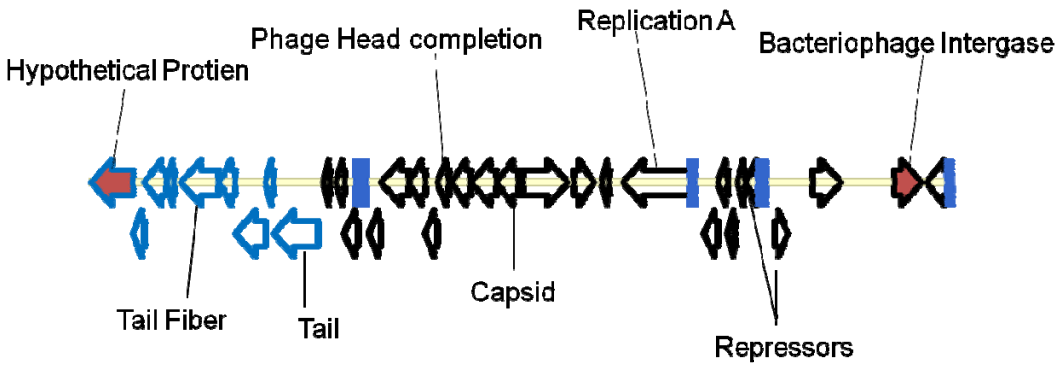

Figure 5. VoGI4 of *V. orientalis*, ORF's in the island outlined by black arrows black arrows. ORF's not calculated to be part of the island outlined in blue. Genes related to phage synthesis are labeled. The flanking genes of phage-related genes are in red.

Table 6. VoGI4 extended to include phage genes and flanking genes.

Locus ID	Product
VIA_001505	hypothetical protein
VIA_001506	hypothetical protein
VIA_001507	hypothetical protein
VIA_001508	hypothetical protein
VIA_001509	Probable tail fiber protein
VIA_001510	hypothetical protein
VIA_001511	Putative bacteriophage protein
VIA_001512	hypothetical protein
VIA_001513	COG5283: Phage-related tail protein
VIA_001514	orf30
VIA_001515	orf29
VIA_001516	putative phage lysozyme
VIA_001517	hypothetical protein
VIA_001518	hypothetical protein
VIA_001519	hypothetical protein
VIA_001520	Conserved hypothetical phage protein
VIA_001521	hypothetical protein
VIA_001522	hypothetical protein
VIA_001523	phage head completion protein (GPL)
VIA_001524	Probable terminase, endonuclease subunit
VIA_001525	Major capsid protein precursor
VIA_001526	Probable capsid scaffolding protein
VIA_001527	Possible [Haemophilus phage HP1] orf16-like phage protein
VIA_001528	phage portal protein, pbsx family
VIA_001529	hypothetical protein
VIA_001530	Replication gene A protein
VIA_001531	hypothetical protein
VIA_001532	COG0030: Dimethyladenosine transferase (rRNA methylation)
VIA_001533	hypothetical protein
VIA_001534	hypothetical protein
VIA_001535	hypothetical protein
VIA_001536	Phage regulatory protein like CII
VIA_001537	hypothetical protein
VIA_001538	hypothetical protein
VIA_001539	putative bacteriophage CI repressor protein
VIA_001540	protein phosphatase 2C domain protein
VIA_001541	Protein kinase
VIA_001542	Protein kinase
VIA_001543	Probable bacteriophage integrase
VIA_001544	hypothetical protein
VIA_001545	Deoxyribodipyrimidine photolyase (EC 4.1.99.3)

3.3.2 Integron

The *V. orientalis* genome contains a gene associated with super-integron structures in *Vibrio* species. The *intI4* gene (VIA_000915), found on the small chromosome, codes for an integrase gene that is part of the super integron cassette in several *Vibrio* species. The integrase gene is one of three key components essential for a functioning integron [26]. The *intI4* gene of *V. orientalis* shows 62.4% nucleotide similarity to the *intI4* gene of *V. cholerae* N16961, 65.1% similarity to the *intIA* gene of *V. parahaemolyticus,* and 68.5% nucleotide similarity to the *intIA* gene of *V. vulnificus* YJ016. Downstream from the *intI4* gene, a conserved cluster of ribosomal genes and the initiation factor, 50S ribosomal protein L20, 50S ribosomal protein L35, and initiation factor IF3 (VIA_000912 to VIA_00914) are found (Fig. 6). This cluster is on the genome of *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* downstream of SI in the three genomes. The presence of the *intI4* gene may be indicative of a super integron system in the *V. orientalis* genome and the ability to incorporate and utilize foreign ORF's for adaptation and a variety of other functions [25-27]. Downstream of the *intI4* gene, there are several ORFs that code for hypothetical proteins, another characteristic of superintegrons which code for a majority of non identifiable genes [108]. When the first 25 kb downstream of *intI4* was searched through NCBI BLAST, a majority of similarities within the NCBI database are from identified genes, but not the hypothetical proteins. Majority of the first 25kb do not show any significant similarity to known sequences in the NCBI database. Analysis is needed on two components that are essential for an integron, the *attI* and attC primary and secondary recombination sites respectively. The

analysis of these sites, which allows expression and integration into the genome, will give more insight into the genes that are in the integron and the size of the integron.

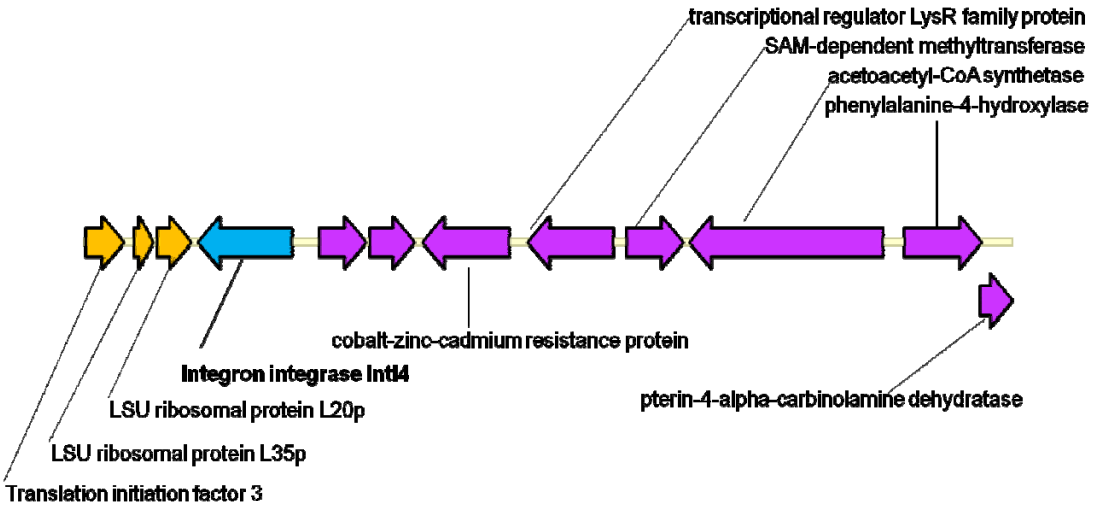

Figure 6. The figure illustrates location of the *intI4* gene in the small chromosome. The three ribosomal related genes are orange. The first eight ORF's are labeled upstream from the *intI4* gene.

3.4 Plasmid Elements

parB is present on both C-I, on ctg78 and C-II. Two plasmid related genes *parD* (VIA_002806) and *parE* (VIA_002805) are located on ctg 80 and compromise the *parDE* operon. The *parDE* operon codes for a toxin-antitoxin (TA) system that has been documented as a region of the broad host range plasmid RK2 and RP4 and codes for two small proteins, ParD and ParE [109]. The two proteins are required for stabilization of the plasmids [109]. The *parE* gene codes for a toxin protein that inhibits cell growth and cell death via cell filamentation, demonstrated by *E. coli* [109]. The *parD* gene codes for antitoxin protein to ParE, preventing its activity and allowing for normal cell growth [109]. The *parDE* operon acts to ensure retention of the RK2 plasmid of the cells by mediating toxic activity and cell killing of daughter cells that lack a plasmid [109]. TA systems have been shown to abundant in free-living prokaryotes, and have been suggested to be linked with pathogenicity of bacteria [110]. The *parDE* operon may or may not be linked with a plasmid element in *V. orientalis*, as seen with other TA systems in prokaryotic cells. Analysis is needed in order to determine whether an RK2 or a related plasmid is stabilized by this operon.

3.5 Virulence Factors

The *V. orientalis* genome contains several commonly characterized virulence factors that are present in virulent *Vibrio* species. Genes coding for hemolysins, lipases, RTX toxins, siderophores, and sialidases are found on the genome on both chromosomes (Table 7). Several putative hemolysins and a hemolysin precursor are present in the genome. This suggests the ability of *V. orientalis* to disrupt epithelial cells and enter the blood stream of its host [5]. In the genome, lipases, phospholipases, and lipase chaperone

genes are present. These genes are linked with disease processes, affecting cellular physiology, and may be linked with cell hemolysins [111]. There are no known illnesses which have been caused by *Vibrio orientalis*. Genes and processes related to virulence can also be linked with enhanced survival in changing environments, ensuring survival in a range of environments that may not be ideal for growth.

Table 7: Some virulence related genes in *V. orientalis*

	Location	Gene
Lipases	VIA_000042	Lysophospholipase L2 (EC 3.1.1.5)
	VIA_000837	Lysophospholipase (EC 3.1.1.5)
	VIA_002638	Lipase chaperone
	VIA_002639	Lipase precursor (EC 3.1.1.3)
	VIA_003192	phospholipase
Hemolysins	VIA_000216	Putative hemolysin
	VIA_000250	Hemolysins and related proteins containing CBS domains
	VIA_000497	Hemolysin
	VIA_000965	Hemolysin
	VIA_001036	putative hemagglutinin/hemolysin-related protein
	VIA_001112	Thermolabile hemolysin precursor
	VIA_001977	Putative hemolysin
	VIA_002221	Hemolysins and related proteins containing CBS domains
	VIA_003159	Hemolysin/cytolysin
	VIA_003847	Putative hemolysin
RTX toxin	VIA_001744	RTX toxins and related Ca2+-binding proteins
Siderophores	VIA_001325	Utilization protein for unknown catechol-siderophore X
	VIA_001472	Ferric siderophore transport system, periplasmic binding protein TonB
	VIA_001473	Ferric siderophore transport system, biopolymer transport protein ExbB
	VIA_001844	Aerobactin siderophore receptor iutA
	VIA_003084	Ferric siderophore transport system, periplasmic binding protein TonB
	VIA_003086	Ferric siderophore transport system, biopolymer transport protein ExbB

3.5.1 Sialic Acid Operon

The sialic acid operon, present on Vibrio Pathogenicity Island (VPI) 2 of *V. cholerae* is also present on the small chromosome of the *V. orientalis* genome (VIA_001776 to VIA_001788). Sialic acid can be utilized as the sole carbon source and components of the operon are vital to the capability of an organism to utilize this acid as a carbon source. Genes responsible for sialic acid metabolism are shown in Figure 7. The operon contains three core enzymes, the *nan* cluster in *V. cholerae*, *E. coli,* and related species [112, 113]. The *nan* cluster consists of the N-acetylneuraminate (NAM) lyase (*nanA*), the NAM kinase (*nanK*), and NAM-6-phosphate epimerase (*nanE*). Minimally, *nanA, nanE, and nanK* make up a complete *nan* system and these genes code for proteins upregulated in the presence of sialic acid to catabolize sialic acid [113]. A lack of evidence exists linking ability of catabolism of sialic acid and its role in pathogenicity of *V. cholerae* in the human host [112]. However, sialic acid catabolism may help *V. cholerae* survive in the gastrointestinal tract and thereby offer a competitive advantage during infection in mucous rich environments. This mechanism may enhance the ability to degrade the mucin layer of the gasterointestinal tract and play a role in pathogenesis of the bacterium in the human host [112, 114]. The tripartite ATP-independent periplasmic (TRAP) transporter genes are also present in the *nan* operon. The TRAP system has been suggested to increase uptake affinity via the periplasmic binding component of the transporter system. Defects in the TRAP genes prevents sialic acid transport [113]. The *V. orientalis* genome possesses an extra copy of the *nanA* gene, however, one copy, VIA_001780 does not show any significant similarity to any sialic acid related genes, and may have been a result of a mis-labeled gene or sequencing error. The two core regions, the TRAP transport system and the *nan* cluster, are shown in Table 8 and Figure 7. The

V. orientalis genome does not have a sialidase gene, as *V. cholerae*; however, there are species that lack this enzyme, yet are able to catabolize sialic acid [113]. The presence of genes related to the sialic acid catabolic pathway, including core genes of the *nan* cluster, and presence of a transmembrane protein, suggests that *V. orientalis* is able to utilize sialic acid as a carbon and energy source, with a selective advantage where sialic acid is available. It also points to *V. orientalis* having a commensal relationship with aquatic animals.

Table 8. Sialic Acid operon in *Vibrio orientalis* and *Vibrio cholerae*.

The genes that are labeled the same gene in *V. orientalis* and *V. cholerae* are highlighted in blue. The different genes are highlighted in pink.

	Location	Gene
V. orientalis	VIA_001776	hypothetical protein
	VIA_001777	Sialic acid-induced transmembrane protein YjhT(NanM), possible mutarotase
	VIA_001778	Sialic acid-induced transmembrane protein YjhT(NanM), possible mutarotase
	VIA_001779	Sialic acid utilization regulator, RpiR family
	VIA_001780	N-acetylneuraminate lyase (EC 4.1.3.3)
	VIA_001781	N-acetylneuraminate lyase (EC 4.1.3.3)
	VIA_001782	TRAP-type transport system, large permease component, predicted N-acetylneuraminate transporter
	VIA_001783	TRAP-type transport system, small permease component, predicted N-acetylneuraminate transporter
	VIA_001784	TRAP-type transport system, periplasmic component, predicted N-acetylneuraminate-binding protein
	VIA_001785	N-acetylmannosamine-6-phosphate 2-epimerase (EC 5.1.3.9)
	VIA_001786	N-acetylmannosamine kinase (EC 2.7.1.60)
	VIA_001787	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)
	VIA_001788	drug resistance transporter, Bcr/CflA subfamily
V. cholerae N16961	VC1748	hypothetical protein
	VC1748	hypothetical protein
	VC1749	Sialic acid-induced transmembrane protein YjhT
	VC1750	Sialic acid-induced transmembrane protein YjhT
	VC1751	Sialic acid utilization regulator, RpiR family
	VC1752	N-acetylneuraminate lyase (EC 4.1.3.3)
	VC1753	TRAP-type transport system, large permease component, predicted N-acetylneuraminate transporter
	VC1754	TRAP-type transport system, small permease component, predicted N-acetylneuraminate transporter
	VC1755	TRAP-type transport system, periplasmic component, predicted N-acetylneuraminate-binding protein
	VC1756	hypothetical protein
	VC1757	N-acetylmannosamine-6-phosphate 2-epimerase (EC 5.1.3.9)

	VC1758	N-acetylmannosamine kinase (EC 2.7.1.60)
	VC1759	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)
	VC1760	Sialidase (EC 3.2.1.18)
	VC1761	Predicted transcriptional regulator
	VC1762	DNA repair protein RadC

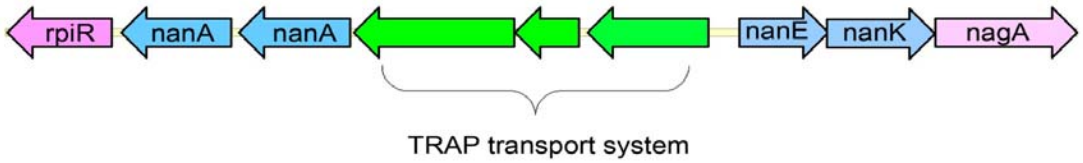

Figure 7. Sialic acid metabolism genes in the *V. orientalis* genome, found on contig 79 on the small chromosome.

3.5.2 Late infection induced genes

Late infection induced genes in *V. cholerae* N16961 are genes induced late in the infection cycle of the bacterium. These genes have been hypothesized to increase fitness of the bacterium when introduced back into the environment [115]. Several genes presented in the study by Lombardo et al. [115] are homologous to genes in the *V. orientalis* genome. The presence of homologous genes in the *V. orientalis* genome may give insight into genes that enhance fitness of *V. orientalis* in the environment. There were no homologous genes to those in *V. cholerae* that increased ability to infect hosts [115]. The homologous genes include those related to metabolism and increased ability to survive in the marine environment after leaving the host environment. One homologous gene, the activator of the cis *dctQM* operon, C4-dicarboxylate transport transcriptional regulatory protein, activates a transporter for malate, succinate and fumerate, enhancing the ability to utilize carbon sources for growth [115]. A gene coding for a glycerol kinase was found and is a key enzyme related to the carbon cycle [115]. The presence of these genes suggest that *V. orientalis* is able to utilize these proteins in the environment, giving insight into the mechanisms it may use [115].

3.5.3 Siderophores

The possession of a sideorophore mediated transport system is related to increased virulence in bacterial pathogens, including *Aeromonas salmonicida, V. anguillarum*, *Salmonella enterica*, and *V. harveyi* infection in vertebrates [116]. Two copies of *tonB* and *exbB* siderophore transport system genes are present in the *V. orientalis* genome on ctg79 and ctg80. The *tonB, exbB, exbD* genes (VIA_1472 to VIA_1474) are found on ctg79 and are required for a high affinity iron transport system

described in *V. cholerae* and *E. coli* [117]. This operon has been linked with the uptake of heme, vibriobactin, and ferrichrome in *V. cholerae* [117]. The genes are involved in coupling energy from the cytoplasm membrane to the outer membrane receptors, and to stabilize the inner membrane [117]. They are linked with ferrous iron and an ABC transporter for iron uptake, but the genome does not contain genes coding for vibriobactin found in other *Vibrio* species [117]. The presence of the siderophore related gene cluster suggests an ability of *V. orientalis* to transport ferrous iron and respond to low iron environments.

3.5.4 Pilus

Pili are hair like filaments that extend from the bacterial surface and allow different species to attach and colonize different niches [118]. Type IV pili (TFP) are the most widespread among bacteria and are the only type of pili found in both Gram-negative and Gram-positive bacteria [118]. Type IV pili promote bacterial attachment and colonization of biotic and abitoic surfaces, and facilitate adhesion by directly or indirectly promoting inter-bacterial interaction and biofilm formation [118]. There are two subtypes of type IV pili, type IVa pili and type IVb. The presence of genes the *pilMNOPQ* gene cluster (VIA_004139 to VIA_004143) on the *V. orientalis* genome are indicative of type IVa pili synthesis by the bacterium, and is one of the most conserved clusters of this type of pili syntehsis [118]. The presence of pilin synthesis related genes and the organization of these genes in the *V. orientalis* genome indicate *V. orientalis* produces type IVa pili and can attach and colonize different niches.

Genes coding for the mannose-sensitive haemagglutinin (MSHA) pilus, associated with several *Vibrio* species and also classified as a type IVa pili, is present in

the *V. orientalis* genome [119]. MSHA pilus plays a role in biofilm formation and colonization in *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* [119-121]. The MSHA gene cluster, *mshQPODCABFGENMJIH* (VIA_004012 to VIA_004012) is found on ctg77 in *V. orientalis.* The gene cluster is required for assembly and secretion of MSHA pili and is organized into two operons, one for structural proteins, and the other for secretion in the *V. cholerae* El Tor genome [121]. In *V. cholerae* El Tor, the MSHA pilus promotes adherence to exoskeletons of planktonic crustaceans and oysters, in addition to host colonization factors, and acts as a receptor for bacteriophage [119]. The *mshA* codes for a major structural subunit of the MSHA pilus and a defect in this gene can render a species unable to form biofilms[121]. The *mshA* is also essential for colonization of *E. scolopes* by *V. fischeri* [122]. The presence of the MSHA gene cluster indicates that *V. orientalis* may be able to assemble and secrete the MSHA pilus. This suggests that the bacterium is able to form biofilms and colonize and adhere to marine organisms, enhancing its survival in the environment.

Another TFP is present in the *V. orientalis* genome, the tight adherence (tad) cluster. The genes are on a genomic island, the widespread colonization island (WCI) [123]. *Tad* related genes are found in genomes of both Gram-negative and Gram-positive bacteria [123]. The tad gene cluster is coded by *tadV/cpaA* to *tadG* (VIA_001291 VIA_001303) on ctg79 of the *V. orientalis* genome and tad related gene clusters were also found on another region of ctg79 and ctg 80. *V. cholerae* and *V. vulnificus* both code for the tad TFP and both gene content and organization are conserved in *V. orientalis,* compared with the tad gene clusters of the two other *Vibrio* species [123]. The tad operon codes for a hypothetical structure of the tad secretion system, and also is required

for assembly of adhesive fimbrial low-molecular weight protein *flp* pili, essential for biofilm formation, colonization, and pathogenesis [123]. The *flp* proteins are coded by these genes form a monophyletic group within the type IVb prepilin genes [123]. The presence of the tad gene cluster, and the same gene content and organization as other species in the *V. orientalis* genome suggests *V. orientalis* may have a functional tad secretion system and the related ability to assemble pili facilitating non-specific binding, enhancing biofilm formation and colonization of surfaces.

3.6 Luminescence

V. orientalis is a luminescent bacterium , as was first described when the species was named [87]. The *lux* operon, as in all other luminescent bacteria, is found in the genome of *V. orientalis*. In addition to the core genes, the *luxH* gene is present on the *V. orientalis* genome. Previously, *luxH* was found only in *V. harveyi,* and not in other *lux* operons of the genus *Vibrio,* with the exception of *V. vulnificus* VVL [81, 84]. The *V. orientalis* genome does not possess regulatory *lux* genes *luxI* and *luxR,* which are found in *V. fischeri lux* operons [81]. The core *lux* genes, *luxC, luxD, luxA, luxB, luxE, luxD,* and *luxG,* present in all luminescent *Vibrio* species, were compared, along with different *lux* accessory genes present on each of the species that possess *lux* operon (Fig. 8) [81]. A phylogenetic tree was constructed, based on core *Vibrio lux* genes to compare *Vibrio* species that possess the *lux* operon (Fig 9). The *V. orientalis lux* operon is most closely related to the *V. harveyi lux* operon and also has the same *lux* operon genes, including the same accessory gene. The *luxG* and *luxH* gene in *V. orientalis,* show 92.3% and 96.6% nucleotide sequence similarity, respectively, to *V. harveyi lux* genes. The close relationship of the *V. harveyi* and *V. orientalis lux* operon supports the hypothesis that the

lux operon is vertically acquired. The presence of a *luxH* gene in the *V. orientalis* genome, not discovered in other *lux* operons, further supports vertical acquisition of the operon coding for luminescence because of similar whole *lux* operons possessed by closely related species. The presence of the *lux* operon in *V. vulnificus* has been suggested to be due to HGT because it is the only strain of the *V. vulnificus* that is described as luminescent and the close evolutionary relationship of the *lux* operon compared with the *lux* operon of *V. harveyi* [86].

Vibrio orientalis contains a *luxO* repressor (VIA_003573) gene and a putative *luxO* gene (VIA_003054). In *V. harveyi*, LuxO represses luminescence at low cell density, and is deactivated in the presence of auto inducers [85]. The *luxO* genes of *V. orientalis* and *V. harveyi* have 75.2% nucleotide similarity. The presence of the *luxO* gene suggests that *V. orientalis* luminescence is under the control of LuxO repression at low cell densities and luminescence is density dependent in *V. orientalis* [85].

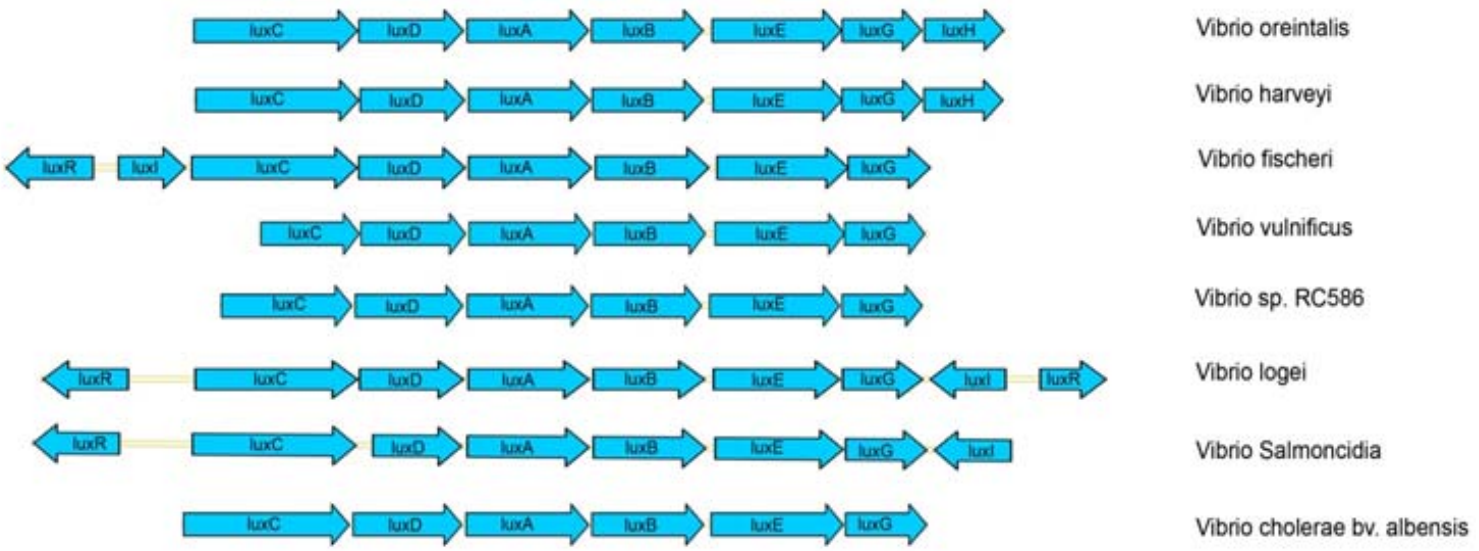

Figure 8. Visual alignment of the lux operon of species compared in the phylogenetic tree. The core genes are aligned in the middle, and different accessory genes are found at the ends of both sides of the operon. Direction of open reading frames are shown by arrows and genes are blue.

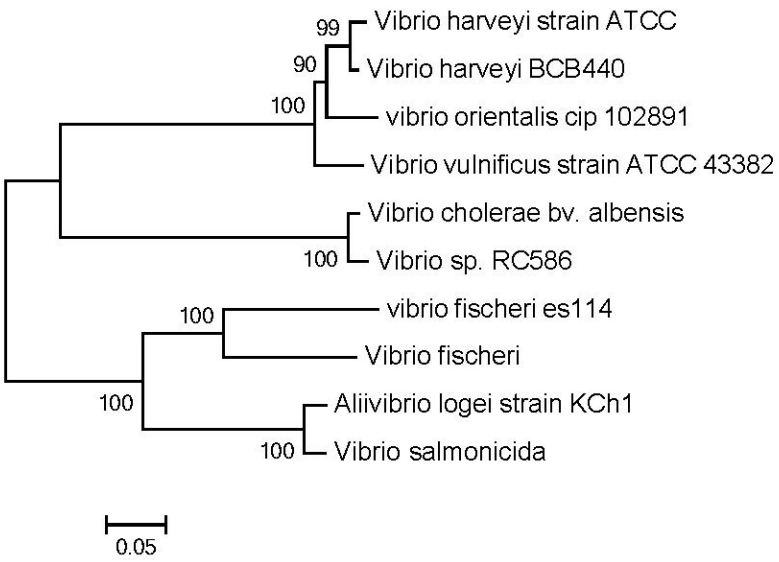

Figure 9. Relationship of the core *lux* operon of *Vibrio* species. The *lux* genes and spacer regions were taken as one sequence and compared. Bootstrap values are labeled.

3.7 Survival in the Environment

Vibrio orientalis was first isolated from the Yellow Sea off the coast of China and grows at 4°C but not at 40°C. A second strain was isolated from shrimp in the same study [87]. Survival mechanisms in response to changing conditions in the environment are advantageous to toxigenic species where these mechanisms may enhance infection. Survival mechanisms include ability to adapt to changing temperature, pH, nutrient levels, and association with marine organisms.

3.7.1 Outer Membrane Proteins

Outer membrane proteins (OMPs) compose about half of the outer membrane mass of Gram-negative bacteria and play a key role in the ability of species to adapt to changing environments. *Vibrio orientalis* codes for an array of OMPs, including the *ompW* precursor, *ompU, ompX, ompK, ompTolC*. *OmpW* precursor is located on ctg79 of the *V. orientalis* genome (VIA_001481). The *ompW* gene codes for an osmotic stress response protein in *V. alginolyticus* and *V. parahaemolyticus.* OmpW protein senses salinity stress in response to changing sodium chloride concentrations in the surrounding environment [124-126]. Both *ompU* and *ompX* are activated in response to low osmolarity [127]. The *ompU* transcription is induced by bile salts in *V. fischeri, V. anguillarum,* and *V. cholerae* and promotes enhanced survival in response to exposure to bile salts, including formation of porins in *V. cholerae* [128, 129]. The OmpU protein also enhances the ability of *V. fisheri* to colonize and form biofilms by *V. anguillarum* [128, 129]. *ompU* is regulated by toxR in *V. cholerae*, and has been suggested to have adhesion properties which may enhance pathogenicity and colonization of the small intestine [130]. The *ompU* genes of *V. orientalis* and *V.*

cholerae N16961 share 78% nucleotide similarity. *ompX* is linked with iron homeostasis in *E. coli* and is also associated with serum resistance and linked with virulence properties [131]. OmpK is a common outer membrane protein present in many *Vibrio* species and has been suggested as a receptor for vibriophage KVP40 in *V. parahaemolyticus* [132]. The *ompK* gene in *V. orientalis* (VIA_003242) has high nucleotide sequence similarity to *ompK* of *V. vulnificus* (82%), *V. parahaemolyticus* (84%), and *V. alginolyticus* (80%). Lastly, the outer membrane protein *tolC* gene is found on ctg80 (VIA_003865). This gene has been linked to bile resistance and colonization inside the intestine and is needed to transport proteins required for RTX secretion [133]. The TolC protein is an exit duct for proteins and drugs and is required for colonization of *V. cholerae [134]*. The presence of outer membrane proteins in the *V. orientalis* genome suggests that *V. orientalis* may have a capability to monitor osmolarity in the environment, transcribe appropriate proteins when exposed to bile salts, and enhance formation of biofilms and colonization of marine animals. Further work is needed to determine which OMPs are upregulated when *V. orientalis* is exposed to high salt concentrations.

3.7.2 Flagellum

Vibrio orientalis possesses a single, sheathed, polar flagellum [87]. Five regions on the *V. orientalis* genome code for polar flagellar proteins, all found on C-I, ctg80. Many genes are conserved, compared with the flagellar genes of *V. cholerae* and *V. parahaemolyticus [135]*. The first region (VIA_003630 to VIA_003647) codes for Region1, and includes genes for basal body rod, rings, hook, ring, and the filament. *flgMN* are the regulatory genes for region 1. The second region codes

(VIA_002333 to VIA_002357) for the basal body, switch, and export, and filament. Region 3 codes for export, regulation, and chemotaxis (VIA_002394 to VIA_002405) and shows the same organization as *V. cholerae* [135]. Region 4 and 5, both code for flagella motor rotation genes, *motAB* (VIA_002298 and VIA_002299*),* and *motY* (VIA_003595) [135, 136], respectively, and are found on different regions of the genome, similar to *V. cholerae* and *V. parahaemolyticus* [135]. The two regions containing flagellin genes, *flaC flaD* (VIA_003631 and VIA_003630) and *flaF flaD flaB* (VIA_002333 to VIA_0023335) are different in *V. orientalis,* compared with flagellin genes in *V. cholerae, V. parahaemolyticus,* and *V. anguillarum,* with regard to content [137]. Like *V. cholerae* and *V. anguillarum, V. orientalis* lacks a third flagellin gene in region 1, and has a total of five flagellin genes, compared with six in *V. parahaemolyticus* [137]. The *flaA* gene is critical for motility in *V. cholerae* as is the *flaC* in *V. parahaemolyticus* [137]. The *flaC* gene in *V. orientalis* may by critical for motility due its location, compared with the *flaC* and *flaA* gene location of *V. parahaemolyticus* and *V. cholerae,* respectively. The organization of genes coding for flagellum biosynthesis in the *V. orientalis* genome are similar to *V. cholerae*, but are different with respect to the flagellin genes and regulatory regions [137]. The genes that code for flagellin, export and regulation are usually species specific, and is the case for *V. orientalis* CIP102891.

3.7.3 Nitrogen and Phosphorus Metabolism

Cycling of carbon, nitrogen and phosphorus are important functions in all *Vibrio* species [4] and *Vibrio orientalis* possess several genes related to nitrogen metabolism. First, exposure to nitric oxide activates a mechanism to detoxify via the nitric oxide reductase operon. The *norR*, *norV*, and *norW* genes are present in tandem on the genome on the small chromosome (VIA_001885 to VIA_001887). *norR* codes for nitric oxide reductase transcriptional regulator. In *E. coli*, the NorR protein senses exposure to nitric oxide, and activates the transcription of *norVW*, together coding for nitric oxide reductase activity [138]. The presence of this operon suggests that *V. orientalis* may be able to survive in the presence of highly reactive nitrogen containing chemicals in the environment. The nitrate reductase gene *napA* is present on the *V. orientalis* genome, coding for a complex in the periplasmic compartment and is widely distributed throughout the genus *Vibrio* [139]. The *napA* gene (VIA_000987) codes for the large subunit of the nitrate reductase complex the presence of nitrate reductase genes indicates *V. orientalis* has a functional nitrate reductase complex in the periplasmic compartment and, thus, is able to reduce nitrate to ammonia, contributing to the nitrogen cycle in the marine environment.

Several subsystems are involved in processing phosphorus, the genes for which are present on the *V. orientalis* genome, including genes for phospholipid synthesis and DNA and RNA synthesis. The inorganic phosphate specific transporter (*pst*) system is coded by *pstSCAB-phoU* (VIA_002173 to VIA_002179) codes for a high affinity inorganic phosphate transport system , and is also a regulator of the *phoBR* complex [140, 141]. The phosphate (*pho*) regulon, present in both *V. cholerae*

and *E. coli* [140, 141], is controlled by the *phoBR* complex (VIA_002171 and VIA_002172) that activates transcription of the Pho regulon, activated in response to low inorganic phosphate, maintaining phosphate homeostatsis and is turned off by high concentrations of inorganic phosphate in the environment [140, 141]. PhoB has also been implicated in controlling expression of virulence genes essential to *V. cholerae*[140]. The *phoB* of *V. orientalis* shows 83% nucleotide similarity to that of *V. cholerae* and 84% nucleotide similarity to *V. vulnificus* and 82% to *V. parahaemolyticus.* The presence of a *pho* regulon indicates that *V. orientalis* is able to transport and sequester inorganic phosphate in response to exposure to low inorganic phosphate environments.

The ability of *V. orientalis* to metabolize these nutrients, and contribute to the nutrient pool gives insight into the role of the bacterium in the environment. However, which compounds are preferentially metabolized by this bacterium remain to be determined.

3.7.4 Utilization of Iron

The *iutA* (VIA_001844) gene is present on the *V. orientalis* genome and is linked with iron uptake and transcribed in response to low iron availability [142, 143]. The *iutA* gene in *V. orientalis* and *V. splendidus* shows 75.5 % nucleotide similarity. The *iutA* genes of *V. vulnificus, V. mimicus, V. furnissii,* and *V. fischeri* all have at least 70% nucleotide similarity to those of *V. orientalis*. The IutA protein is an outer membrane protein that functions as a ferric aerobactin receptor and is important for utilization of ferric aerobactin in iron limiting conditions in *Vibrio* species and *E. coli* [142]. The *iutA* gene is found in tandem to ferric aerobactin

transporter genes (VIA_001840 to VIA_001842). The presence of *iutA* genes and transporter genes suggest an ability of *V. orientalis* to uptake and use aerobactin in iron limiting conditions and thereby survive and grow under iron limiting conditions.

Another gene related to iron sensing is *fur,* which codes the ferric uptake regulation (Fur) protein. The *fur* gene in *Vibrio orientalis* is found on ctg80 (VIA_002272) and shows at least 80% nucleotide similarity to that of several *Vibrio* species, including *V. furnissii, V. cholerae, V. anguillarum, V. vulnificus, V. harveyi, V. alginolyticus, and V. splendidus*. Fur acts as a repressor and coordinates transcription of various genes involved in maintaining iron homeostasis, including those related to iron uptake, metabolism, and storage, which are critical for growth. Fur also prevents iron toxicity [144]. Fur is deactivated under low iron condition and genes involved in uptake and metabolism of iron are activated [144]. Regulation, transport and maintenance of iron levels are vital functions. Hence, presence of *fur* in *V. orientalis* suggests it is able to protect itself from iron toxicity and the transcription is coordinated by the amount of iron in the environment, activating iron uptake, storage and metabolism, supporting adaptation of *V. orientalis* to iron limiting conditions.

3.7.5 Chitin Digestion

Four copies of chitinases are present on the *V. orientalis* genome. As with many *Vibrio* species, *V. orientalis* contains genes that code for chitnolytic enzymes that play an important role in the breakdown of chitin in the marine environment and the recycling of insoluble carbon and nitrogen [145]. Chitinases are crucial in colonization of several *Vibrio* species on surfaces of crustaceans and chitinous

zooplankton [48, 145]. *Vibrio* species are able to utilize chitin as a primary carbon and nitrogen source [145, 146]. The breakdown of chitin is a complex process involving sensing, attachment, transport, and catabolism [3]. The presence of genes encoding chitinases suggests that *V. orientalis* successfully associates with chitinous organisms, including zooplankton and crustaceans, breaking down the chitin when the population blooms crash, thus contributing to the recycling of inorganic carbon and nitrogen, by utilizing chitin as a carbon and nitrogen source. The association with chitinous organisms is an evolved function of *V. orientalis* in its mutual role in the environment.

3.7.6 Response to environmental stressors

Environmental stressors including pH, temperature, grazing and nutrient limitation, influence the natural functions of *Vibrio* species [1]. They must adapt to changing environments and have evolved several response mechanisms to combat stress when these changes affect the growth and survival of the organisms. First, the *V. orientalis* genome contains a multiple stress response protein, *rpoS* (VIA_000249), or alternative sigma factor. The gene shows 77% nucleotide similarity to the same gene in the *V. harveyi* genome, 74% similarity to *V. cholerae,* and 76% similarity to *V. vulnificus.* RpoS regulates stationary phase response and stress response genes including those involved in starvation, osmotic stress, temperature, and pH changes [147]. In *Vibrio* species, response to different stressors is species specific, with other response mechanisms in addition to RpoS regulation [147-149]. In *V. harveyi,* the *rpoS* protein controls response to ethanol changes [149]. RpoS stress regulator works in *V. cholerae* in response to hyperosmotic changes and nutrient deprivation and in *V.*

vulnificus exposure to hydrogen peroxide hyperosmolarity and acidic conditions [147-149]. The *rpoS* of *V. orientalis* may allow the organism to respond to stressors that include changes in osmotic, ethanol, and nutrient pressures; however, specific functions of RpoS in *V orientalis* remain to be defined.

3.7.6.1 Response to pH

The *V. orientalis* genome contains several genes related to response to pH. The *cad* operon, associated with acid stress, is located on ctg78 (VIA_000489 to VIA_00491) in its genome and functions to counteract acidification in the external environment. It is present in *V. cholerae, V. parahaemolyticus, V. vulnificus,* and other *Vibrio* species [61, 62, 150]. *cadA* codes for lysine decarboxolase in *V. orientalis* and is induced when acidification occurs in the external environment [150]. CadC is responsible for pH regulated expression of *cadBA* genes through activation of a transcriptional promoter not part of the operon [151]. The *cadA* gene in *V. orientalis* shows at least 77% nucleotide similarity to *cadA* genes in *V. cholerae* (79.4%), *V. parahaemolyticus* (77.8%), *and V. vulnificus* (79.2%).

Under alkaline conditions, Na+/H+ antiporters help maintain homeostasis of bacterial cells [152]. The *V. orientalis* genome contains *nhaA* (VIA_003122), *nhaC* (VIA_003518) and *nhaD* (VIA_001677) genes that code Na+/H+ antiproters. The NhaA antiporter enhances ability to survive under alkaline conditions in *V. cholerae, E. coli,* and *V. parahaemolyticus.* The antiporter is activated when the pH is increased [152, 153]. The *nhaA* gene in *V. orientalis* shows 75% similarity with *nhaA* of *V. parahaemolyticus,* and 72% to *V. cholerae and V. vulnificus.* NhaD antiporter is activated and has the highest level of activity at pH 8.5 to 9 [154]. The *nhaD* gene of

V. orientalis has 72% similarity to *nhaD* in *V. parahaemolyticus.* A second type of antiporter family gene is found on the *V. orientalis* genome, the *nhaP* gene (VIA_002009). The NhaP Na+/H+ and K+/H+ antiporter is activated under alkaline conditions optimally with K+ accumulation and a pH of approximately 7.75 in *V. cholerae* [155]. The antiporter is also able to work as an Na+/H+ antiporter in the absence of K+ [155]. The *nhaP* gene in *V. orientalis* shows 65% and 66% nucleotide similarity to *V. vulnificus* and *V.* cholerae, respectively.

3.7.6.2 Temperature

Temperature is a major factor affecting the ecology of *Vibrio* species, influencing the ability of *Vibrio* spp. to perform basic cell functions. Cold shock proteins are induced when a cell is exposed to low temperatures and these include *cspA* (VIA_001847), *cspD* (VIA_002501), and *cspE* (VIA_002715*)*. They are present on the small chromosome of *V. orientalis*. Expression of *cspA* and *cspD* is induced when *V. vulnificus* is exposed to cold temperatures [156]. CspA is a key element in triggering the cold shock response [156]. The *cspA* of *V. orientalis* and *V. vulnificus* have 75% nucleotide similarity. A second cold shock protein gene cluster, paraquat-inducible protein A and B (VIA_002969 and VIA_002968, respectively), is present on ctg80 of the *V. orientalis* genome. This gene cluster is upregulated during cold shock in *V. parahaemolyticus* [59]. Expression of paraquat-inducible protein A is gradually upregulated when cells are exposed to increasingly colder temperatures, while paraquat-inducible protein B is upregulated and stays at the same expression level while temperatures decrease [59]. The paraquat-inducible A and B genes are present in several *Vibrio* species, including *V. vulnificus, V. parahaemolyticus,* and *V.*

alginolyticus. *V. vulnificus* and *V. parahaemolyticus* show 65% similarity to *V. orientalis,* with respect to these two genes.

The *V. orientalis* genome contains several genes that code for heat shock proteins, including *groEL* (VIA_000501), *groES* (VIA_000502), *dnaK* (VIA_002241) and *dnaJ* (VIA_002242). The *rpoH* (VIA_000023) gene encodes a promoter that is highly expressed under heat shock and transcribes downstream heat shock proteins [157]. RpoH controls activation of the chaperone genes, g*roES* and *dnaK*, of the groEL-groES and dnaK-dnaJ clusters that are overproduced when exposed to higher temperatures and play a major role in preventing intracellular protein aggregation in *V. cholerae* [158]. When exposed to a temperature increase of 7°C, the genes are induced [158]. The two chaperone genes g*roES* and *dnaK* of *V. orientalis* have high nucleotide similarity, ≥ 84% and 83% respectively, to several *Vibrio* species, including *V. cholerae, V. parahaemolyticus, V. harveyi, V. fischeri* and *V. vulnificus.*

3.7.7 Quorum Sensing

The ability to quorum sense is important under environmental conditions, activating adaptive responses, coordinated expression of virulence, bioluminescence, and biofilm formation in *Vibrio* species [159]. In the *V. orientalis* genome, genes linked with quorum sensing on the genome include *luxP* (VIA_001151), *luxQ* (VIA_001152), *luxS* (VIA_003963), *luxO (*VIA_003573) and (VIA_003054), *luxU*(VIA_003574), and *hapR*(VIA_000273). The *luxS* gene is required for production of AI-2, and can detect density of populations around the cell, with the capability of sensing both intracellular and intercellular density [65, 159]. LuxP

detects AI-2, and causes changes in conformation of the LuxQ protein. Change in LuxQ triggers a signaling cascade related to removal of the phosphate group attached to LuxO and deactivates the protein, activating transcription of biofilm formation and bioluminescence genes [65]. HapR controls transcription of virulence factors and represses biofilm formation in low cell density environments [159].

Chapter 4 Conclusion

Comparative genomics has permitted exploration of the *V. orientalis* genome, and thereby its characteristics, functions, and features. The relationship of *V. orientalis* to other species has been be elucidated, notably by using the phylogenies that were constructed with homologous genes. Genes acquired via horizontal gene transfer provide insight into possible functions the mobile elements can provide, especially adaptive functions. Genes related to adaptive functions give insight into the environments to which the bacterium is able to adapt, including temperature and salinity regimes. The presence of genes related to colonization of potential hosts provides evidence for commensal relationships.

Although the genome provides insight into metabolic functions and genetic organization of *V. orientalis,* the most valuable insight provided is that associated with the ecology of this bacterium in its natural environment. These insights may enhance its identification from environments, and provide identification where the bacterium may not have been identified previously.

V. orientalis possess genes related with pathogenic potential, and these genes may give insight into the environmental function of the bacterium. Genes such as those related with nitric oxide metabolism may suggest that *V. orientalis* may grow in volatile environments. The presence of the sialic acid operon and various virulence genes that enhance host survival may be related environments that *V. orientalis* may be found. Although the bacterium has not been linked with infections, the presence of these virulence genes points to exploration of how these genes offer an advantage to possess and retain the genes in the genome.

References

1. Thompson FL, Iida T, Swings J: **Biodiversity of vibrios**. *Microbiol Mol Biol Rev* 2004, **68**(3):403-431.
2. Garrity GM, Bell JA, Lilburn TG (eds.): **Taxonomic Outline of the Prokaryotes Release 5.0 Bergey's manual® of systematic bacteriology**, 2nd edn. New York: Springer; 2005.
3. Urakawa H, Rivera I: **Aquatic Environment** *The Biology of Vibrios* 2006:175-189.
4. Thompson J, Polz MF: **Dynamics of Vibrio Populations and Thier Role in Environmental Nutrient Cycling**. *The Biology of Vibrios* 2006:190-203.
5. Dziejman M, Yildiz FH: **Genomics of Pathogenic *Vibrio* Species**. In. Edited by Wiedmann M, Zhang W: Springer New York; 2011: 267-310.
6. Ruby EG: **Lessons from a cooperative, bacterial-animal association: the Vibrio fischeri-Euprymna scolopes light organ symbiosis**. *Annu Rev Microbiol* 1996, **50**:591-624.
7. Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR: **Ecological relationships between Vibrio cholerae and planktonic crustacean copepods**. *Appl Environ Microbiol* 1983, **45**(1):275-283.
8. Okada K, Iida T, Kita-Tsukamoto K, Honda T: **Vibrios Commonly Possess Two Chromosomes**. *Journal of Bacteriology* 2005, **187**(2):752-757.
9. Gomez-Gil B, Roque A: **Isolation, Enumeration, and Preservation of the *Vibrionaceae***. *The Biology of Vibrios* 2006:15-24.
10. Trucksis M, Michalski J, Deng YK, Kaper JB: **The Vibrio cholerae genome contains two unique circular chromosomes**. *Proc Natl Acad Sci U S A* 1998, **95**(24):14464-14469.
11. Yamaichi Y, Iida T, Park KS, Yamamoto K, Honda T: **Physical and genetic map of the genome of Vibrio parahaemolyticus: presence of two chromosomes in Vibrio species**. *Mol Microbiol* 1999, **31**(5):1513-1521.
12. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L *et al*: **DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae***. *Nature* 2000, **406**:477-483.
13. Iida T, Kurokawa K: **Comparative Genomics: Genome Configuration and the Driving Forces in the Evolution of Vibrios**. *The Biology of Vibrios* 2006:67-75.
14. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, Taviani E, Jeon YS, Kim DW, Brettin TS *et al*: **Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic Vibrio cholerae**. *Proc Natl Acad Sci U S A* 2009, **106**(36):15442-15447.
15. Shekar M, Acharya S, Karunasagar I: **Variable repeat regions in the genome of Vibrio vulnificus and polymorphism in one of the loci in strains isolated from oysters**. *Int J Food Microbiol* 2008, **123**(3):240-245.
16. Blackstone GM, Nordstrom JL, Vickery MC, Bowen MD, Meyer RF, DePaola A: **Detection of pathogenic Vibrio parahaemolyticus in oyster enrichments by real time PCR**. *J Microbiol Methods* 2003, **53**(2):149-155.

17. Panicker G, Vickery MC, Bej AK: **Multiplex PCR detection of clinical and environmental strains of Vibrio vulnificus in shellfish**. *Can J Microbiol* 2004, **50**(11):911-922.
18. Haley B, Grim C, Hasan N, Choi S-Y, Chun J, Brettin T, Bruce D, Challacombe J, Detter JC, Han C *et al*: **Comparative genomic analysis reveals evidence of two novel Vibrio species closely related to V. cholerae**. *BMC Microbiology* 2010, **10**(1):154.
19. Lin B, Wang Z, Malanoski AP, O'Grady EA, Wimpee CF, Vuddhakul V, Alves Jr N, Thompson FL, Gomez-Gil B, Vora GJ: **Comparative genomic analyses identify the Vibrio harveyi genome sequenced strains BAA-1116 and HY01 as Vibrio campbellii**. *Environmental Microbiology Reports* 2010, **2**(1):81-89.
20. Baumann P, Baumann L, Woolkalis MJ, Bang SS: **Evolutionary relationships in vibrio and Photobacterium: a basis for a natural classification**. *Annu Rev Microbiol* 1983, **37**:369-398.
21. Boto L: **Horizontal Gene Transfer in Evolution**. In: *eLS*. John Wiley & Sons, Ltd; 2010.
22. Boucher Y, Stokes HW: **The Roles of Lateral Gene Transfer and Vertical Descent in Vibrio Evolution** *The Biology of Vibrios* 2006:84-94.
23. Waldor M, Mekalanos J: **Lysogenic conversion by a filamentous phage encoding cholera toxin.** *Science* 1996, **272**:1910-1914.
24. Boucher Y, Nesbo CL, Joss MJ, Robinson A, Mabbutt BC, Gillings MR, Doolittle WF, Stokes HW: **Recovery and evolutionary analysis of complete integron gene cassette arrays from Vibrio**. *BMC Evol Biol* 2006, **6**:3.
25. Mazel D: **Integrons: agents of bacterial evolution**. *Nat Rev Micro* 2006, **4**(8):608-620.
26. Mazel D, Dychinco B, Webb VA, Davies J: **A distinctive class of integron in the Vibrio cholerae genome**. *Science* 1998, **280**(5363):605-608.
27. Rowe-Magnus DA, Guerout AM, Ploncard P, Dychinco B, Davies J, Mazel D: **The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons**. *Proc Natl Acad Sci U S A* 2001, **98**(2):652-657.
28. Waldor MK, Friedman DI, Adhya S: **Phages: their role in bacterial pathogenesis and biotechnology**. *ASM Press* 2005.
29. Brussow H, Canchaya C, Hardt WD: **Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion**. *Microbiol Mol Biol Rev* 2004, **68**(3):560-602.
30. Iida T, Hattori A, Tagomori K, Nasu H, Naim R, Honda T: **Filamentous phage associated with recent pandemic strains of Vibrio parahaemolyticus**. *Emerg Infect Dis* 2001, **7**(3):477-478.
31. Oakey HJ, Cullen BR, Owens L: **The complete nucleotide sequence of the Vibrio harveyi bacteriophage VHML**. *J Appl Microbiol* 2002, **93**(6):1089-1098.
32. Faruque SM, Rahman MM, Asadulghani, Nasirul Islam KM, Mekalanos JJ: **Lysogenic conversion of environmental Vibrio mimicus strains by CTXPhi**. *Infect Immun* 1999, **67**(11):5723-5729.

33. Grim C, Choi J, Chun J, Jeon YS, Taviani E, Hasan NA, Haley B, Huq A, Colwell RR: **Occurrence of Vibrio cholerae 7th pandemic VSP-I island and a new variant**. *OMICS* 2009, **14**(1):1-7.
34. Faruque S, Albert M, Mekalanos J: **Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae.*** *Microbiol Mol Biol Rev* 1998, **62**:1301-1314.
35. Hurley CC, Quirke A, Reen FJ, Boyd EF: **Four genomic islands that mark post-1995 pandemic Vibrio parahaemolyticus isolates**. *BMC Genomics* 2006, **7**:104.
36. Quirke AM, Reen FJ, Claesson MJ, Boyd EF: **Genomic island identification in Vibrio vulnificus reveals significant genome plasticity in this human pathogen**. *Bioinformatics* 2006, **22**(8):905-910.
37. Cohen AL, Oliver JD, DePaola A, Feil EJ, Boyd EF: **Emergence of a virulent clade of Vibrio vulnificus and correlation with the presence of a 33-kilobase genomic island**. *Appl Environ Microbiol* 2007, **73**(17):5553-5565.
38. Langille M, Hsiao W, Brinkman F: **Evaluation of genomic island predictors using a comparative genomics approach**. *BMC Bioinformatics* 2008, **9**(1):329.
39. Waack S, Keller O, Asper R, Brodag T, Damm C, Fricke W, Surovcik K, Meinicke P, Merkl R: **Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models**. *BMC Bioinformatics* 2006, **7**(1):142.
40. Vezzulli L, Pezzati E, Moreno M, Fabiano M, Pane L, Pruzzo C: **Benthic ecology of Vibrio spp. and pathogenic Vibrio species in a coastal Mediterranean environment (La Spezia Gulf, Italy)**. *Microbial Ecology* 2009, **58**(4):808-818.
41. Heidelberg J, Heidelberg K, Colwell R: **Seasonality of Chesapeake Bay bacterioplankton species**. *Appl Environ Microbiol* 2002, **68**(11):5488-5497.
42. Gil A, Huq A, Lanata CF, Taylor DN, Orozco R, I.N.G. R, Patz J, Colwell RR: **Environmental sources of *Vibrio cholerae* on the Peruvian Coast.** In: *US-Japan Cholera Conference: December 2-5, 1999 1999; Baltimore, Maryland*; 1999.
43. Oliver JD, Warner RA, Cleland DR: **Distribution and ecology of Vibrio vulnificus and other lactose-fermenting marine vibrios in coastal waters of the southeastern United States**. *Appl Environ Microbiol* 1982, **44**(6):1404-1414.
44. Pfeffer CS, Hite MF, Oliver JD: **Ecology of Vibrio vulnificus in estuarine waters of eastern North Carolina**. *Appl Environ Microbiol* 2003, **69**(6):3526-3531.
45. Louis V, Russek-Cohen E, Choopun N, Rivera I, Gangle B, Jiang S, Rubin A, Patz J, Huq A, Colwell R: **Predictability of *Vibrio cholerae* in Chesapeake Bay**. *Appl Environ Microbiol* 2003, **69**(5):2773-2785.
46. Randa MA, Polz MF, Lim E: **Effects of temperature and salinity on Vibrio vulnificus population dynamics as assessed by quantitative PCR**. *Appl Environ Microbiol* 2004, **70**(9):5469-5476.

47. Svitil AL, Chadhain S, Moore JA, Kirchman DL: **Chitin Degradation Proteins Produced by the Marine Bacterium Vibrio harveyi Growing on Different Forms of Chitin**. *Appl Environ Microbiol* 1997, **63**(2):408-413.
48. Kaneko T, Colwell R: **Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay.** *J Bacteriol* 1973, **113**(1):24-32.
49. Huq A, Small E, West P, Huq M, Rahman R, Colwell R: **Ecological relationship between *Vibrio cholerae* and planktonic copepods.** *Appl Environ Microbiol* 1983, **45**:275-283.
50. Heidelberg J, Heidelberg K, Colwell R: **Bacteria of the gamma-subclass Proteobacteria associated with zooplankton in Chesapeake Bay**. *Appl Environ Microbiol* 2002, **68**(11):5498-5507.
51. Ruby EG: **The Euprymna scolopes-Vibrio fischeri symbiosis: a biomedical model for the study of bacterial colonization of animal tissue**. *J Mol Microbiol Biotechnol* 1999, **1**(1):13-21.
52. Visick KL, McFall-Ngai MJ: **An exclusive contract: specificity in the Vibrio fischeri-Euprymna scolopes partnership**. *J Bacteriol* 2000, **182**(7):1779-1787.
53. McFall-Ngai M: **Host-microbe symbiosis: the squid-Vibrio association--a naturally occurring, experimental model of animal/bacterial partnerships**. *Adv Exp Med Biol* 2008, **635**:102-112.
54. Benitez JA, Silva AJ, Finkelstein RA: **Environmental signals controlling production of hemagglutinin/protease in Vibrio cholerae**. *Infect Immun* 2001, **69**(10):6549-6553.
55. Liu MC, Bakel BW, Liu MY, Dao TN: **Purification of Vibrio fischeri nitrite reductase and its characterization as a hexaheme c-type cytochrome**. *Arch Biochem Biophys* 1988, **262**(1):259-265.
56. Li X, Roseman S: **The chitinolytic cascade in Vibrios is regulated by chitin oligosaccharides and a two-component chitin catabolic sensor/kinase**. *Proc Natl Acad Sci U S A* 2004, **101**(2):627-631.
57. McDougald D, Kjelleberg S: **Adaptive Responses of Vibrios**. *The Biology of Vibrios* 2006:133 - 155.
58. Thompson J, Randa M, Marcelino L, Tomita-Mitchell A, Lim E, Polz M: **Diversity and dynamics of a North Atlantic coastal Vibrio community**. *Appl Environ Micbrobiol* 2004, **70**:4103-4110.
59. Yang L, Zhou D, Liu X, Han H, Zhan L, Guo Z, Zhang L, Qin C, Wong H, Yang R: **Cold-induced gene expression pro¢les ofVibrio parahaemolyticus : a time-course analysis**. *FEMS Microbiology Letters* 2008, **291(2009)**:50-58.
60. Stewart GR, Young DB: **Heat-shock proteins and the host–pathogen interaction during bacterial infection**. *Curr Opin Immunol* 2004, **2004**(16):506-510.
61. Rhee JE, Rhee JH, Ryu PY, Choi SH: **Identification of the cadBA operon from Vibrio vulnificus and its influence on survival to acid stress**. *FEMS Microbiol Lett* 2002, **208**(2):245-251.
62. Tanaka Y, Kimura B, Takahashi H, Watanabe T, Obata H, Kai A, Morozumi S, Fujii T: **Lysine decarboxylase of Vibrio parahaemolyticus: kinetics of**

transcription and role in acid resistance. *J Appl Microbiol* 2008, **104**(5):1283-1293.
63. Pruzzo C, Vezzulli L, Colwell RR: **Global impact of Vibrio cholerae interactions with chitin**. *Environ Microbiol* 2008, **10**(6):1400-1410.
64. Xavier KB, Bassler BL: **LuxS quorum sensing: more than just a numbers game**. *Current opinion in microbiology* 2003, **6**(2):191-197.
65. Henke JM, Bassler BL: **Three parallel quorum-sensing systems regulate gene expression in Vibrio harveyi**. *J Bacteriol* 2004, **186**(20):6902-6914.
66. Miller MB, Bassler BL: **Quorum sensing in bacteria**. *Annu Rev Microbiol* 2001, **55**:165-199.
67. Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL: **Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae**. *Cell* 2002, **110**(3):303-314.
68. Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL: **The major Vibrio cholerae autoinducer and its role in virulence factor production**. *Nature* 2007, **450**(7171):883-886.
69. Xavier KB, Bassler BL: **LuxS quorum sensing: more than just a numbers game**. *Curr Opin Microbiol* 2003, **6**(2):191-197.
70. Myhr E, Larsen JL, Lillehaug A, Gudding R, Heum M, Hastein T: **Characterization of Vibrio anguillarum and closely related species isolated from farmed fish in Norway**. *Appl Environ Microbiol* 1991, **57**(9):2750-2757.
71. Clarridge JE, Zighelboim-Daum S: **Isolation and characterization of two hemolytic phenotypes of Vibrio damsela associated with a fatal wound infection**. *J Clin Microbiol* 1985, **21**(3):302-306.
72. Love M, Teebken-Fisher D, Hose JE, Farmer JJ, 3rd, Hickman FW, Fanning GR: **Vibrio damsela, a Marine Bacterium, Causes Skin Ulcers on the Damselfish Chromis punctipinnis**. *Science* 1981, **214**(4525):1139-1140.
73. Austin B, Zhang XH: **Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates**. *Lett Appl Microbiol* 2006, **43**(2):119-124.
74. Bej AK, Patterson DP, Brasher CW, Vickery MC, Jones DD, Kaysner CA: **Detection of total and hemolysin-producing Vibrio parahaemolyticus in shellfish using multiplex PCR amplification of tl, tdh and trh**. *J Microbiol Methods* 1999, **36**(3):215-225.
75. Igbinosa EO, Okoh AI: **Emerging Vibrio species: an unending threat to public health in developing countries**. *Res Microbiol* 2008, **159**(7-8):495-506.
76. Faruque SM, Mekalanos JJ: **Pathogenicity islands and phages in Vibrio cholerae evolution**. *Trends Microbiol* 2003, **11**(11):505-510.
77. Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond RM, Thompson S, Wilson S, Bean NH, Griffin PM *et al*: ***Vibrio parahaemolyticus* infections in the United States, 1973-1998**. *J Infect Dis* 2000, **181**(5):1661-1666.
78. Yeung PS, Boor KJ: **Epidemiology, pathogenesis, and prevention of foodborne Vibrio parahaemolyticus infections**. *Foodborne Pathog Dis* 2004, **1**(2):74-88.

79. Gulig PA, Bourdage KL, Starks AM: **Molecular Pathogenesis of Vibrio vulnificus**. *J Microbiol* 2005, **43 Spec No**:118-131.
80. Strom MS, Paranjpye RN: **Epidemiology and pathogenesis of Vibrio vulnificus**. *Microbes Infect* 2000, **2**(2):177-188.
81. Meighen EA: **Bacterial bioluminescence: organization, regulation, and application of the lux genes**. *Faseb J* 1993, **7**(11):1016-1022.
82. Fidopiastis PM, von Boletzky S, Ruby EG: **A new niche for Vibrio logei, the predominant light organ symbiont of squids in the genus Sepiola**. *J Bacteriol* 1998, **180**(1):59-64.
83. Stevens AM, Greenberg EP: **Quorum sensing in Vibrio fischeri: essential elements for activation of the luminescence genes**. *J Bacteriol* 1997, **179**(2):557-562.
84. Meighen EA: **Genetics of bacterial bioluminescence**. *Annu Rev Genet* 1994, **28**:117-139.
85. Freeman JA, Bassler BL: **A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in Vibrio harveyi**. *Mol Microbiol* 1999, **31**(2):665-677.
86. Urbanczyk H, Ast JC, Kaeding AJ, Oliver JD, Dunlap PV: **Phylogenetic Analysis of the Incidence of lux Gene Horizontal Transfer in Vibrionaceae**. *J Bacteriol* 2008, **190**(10):3494-3504.
87. Yang Y, Yeh Lp, Cao Y, Baumann L, Baumann P, Tang JS-e, Beaman B: **Characterization of marine luminous bacteria isolated off the Coast of China and description of<i>Vibrio orientalis</i> sp. nov**. *Current Microbiology* 1983, **8**(2):95-100.
88. Ruimy R, Breittmayer V, Elbaze P, Lafay B, Boussemart O, Gauthier M, Christen R: **Phylogenetic analysis and assessment of the genera Vibrio, Photobacterium, Aeromonas, and Plesiomonas deduced from small-subunit rRNA sequences**. *Int J Syst Bacteriol* 1994, **44**(3):416-426.
89. Zenno S, Saigo K: **Identification of the genes encoding NAD(P)H-flavin oxidoreductases that are similar in sequence to Escherichia coli Fre in four species of luminous bacteria: Photorhabdus luminescens, Vibrio fischeri, Vibrio harveyi, and Vibrio orientalis**. *J Bacteriol* 1994, **176**(12):3544-3551.
90. Arias CR, Macian MC, Aznar R, Garay E, Pujalte MJ: **Low incidence of Vibrio vulnificus among Vibrio isolates from sea water and shellfish of the western Mediterranean coast**. *Journal of Applied Microbiology* 1999, **86**(1):125-134.
91. Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formsma K, Gerdes S, Glass E, Kubal M *et al*: **The RAST Server: Rapid Annotations using Subsystems Technology**. *BMC Genomics* 2008, **9**(1):75.
92. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S *et al*: **Geneious v5.4**. 2011.
93. Konstantinidis KT, Tiedje JM: **Genomic insights that advance the species definition for prokaryotes**. *Proc Natl Acad Sci U S A* 2005, **102**(7):2567-2572.

94. Richter M, Rosselló-Móra R: **Shifting the genomic gold standard for the prokaryotic species definition**. *Proceedings of the National Academy of Sciences* 2009.
95. Langille MGI, Brinkman FSL: **IslandViewer: an integrated interface for computational identification and visualization of genomic islands**. *Bioinformatics* 2009, **25**(5):664-665.
96. Thompson JD, Higgins DG, Gibson TJ: **CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice**. *Nucleic Acids Res* 1994, **22**(22):4673-4680.
97. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S: **MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods**. *Molecular Biology and Evolution* 2011, **28**(10):2731-2739.
98. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees**. *Molecular biology and evolution* 1987, **4**(4):406-425.
99. Kimura M: **A Simple Method for Estimating Evolutionary Rates of Base Substitutions through Comparative Studies of Nucleotide-Sequences**. *Journal of Molecular Evolution* 1980, **16**(2):111-120.
100. Klappenbach JA, Dunbar JM, Schmidt TM: **rRNA Operon Copy Number Reflects Ecological Strategies of Bacteria**. *Applied and Environmental Microbiology* 2000, **66**(4):1328-1333.
101. Naka H, Dias GM, Thompson CC, Dubay C, Thompson FL, Crosa JH: **Complete Genome Sequence of the Marine Fish Pathogen Vibrio anguillarum Harboring the pJM1 Virulence Plasmid and Genomic Comparison with Other Virulent Strains of V. anguillarum and V. ordalii**. *Infection and Immunity* 2011, **79**(7):2889-2900.
102. Sozhamannan S, Yildiz FH: **Diversity and Genetic Basis of Polysaccharide Biosynthesis in *Vibrio cholerae* Epidemiological and Molecular Aspects on Cholera**. In. Edited by Ramamurthy T, Bhattacharya SK: Springer New York; 2011: 129-160.
103. Yamasaki S, Shimizu T, Hoshino K, Ho ST, Shimada T, Nair GB, Takeda Y: **The genes responsible for O-antigen synthesis of vibrio cholerae O139 are closely related to those of vibrio cholerae O22**. *Gene* 1999, **237**(2):321-332.
104. Manning PA, Stroeher UH, Morona R: **Molecular basis for O-antigen biosynthesis in Vibrio cholerae O1: Ogawa-Inaba switching**. In: *Vibrio cholerae and cholera: molecular to global perspectives.* Edited by Wachsmuth IK, Blake PA, (ed.) OO. Washington, DC: ASM Press; 1994.
105. Stroeher UH, Karageorgos LE, Morona R, Manning PA: **In Vibrio cholerae serogroup O1, rfaD is closely linked to the rfb operon**. *Gene* 1995, **155**(1):67-72.
106. Reidl J, Mekalanos JJ: **Characterization of Vibrio cholerae bacteriophage K139 and use of a novel mini-transposon to identify a phage-encoded virulence factor**. *Mol Microbiol* 1995, **18**(4):685-701.

107. Paul JH, Sullivan MB: **Marine phage genomics: what have we learned?** *Current Opinion in Biotechnology* 2005, **16**(3):299-307.
108. Rowe-Magnus DA, Zouine M, Mazel D: **The Adaptive Genetic Arsenal of Pathogenic Vibrio Species: the Role of Integrons**. *The Biology of Vibrios* 2006:95-111.
109. Roberts RC, Ström AR, Helinski DR: **The parDE Operon of the Broad-host-range Plasmid RK2 Specifies Growth Inhibition Associated with Plasmid Loss**. *Journal of Molecular Biology* 1994, **237**(1):35-51.
110. Yamaguchi Y, Park J-H, Inouye M: **Toxin-Antitoxin Systems in Bacteria and Archaea**. *Annual Review of Genetics* 2011, **45**(1):61-79.
111. Kang JH, Lee JH, Park JH, Huh SH, Kong IS: **Cloning and identification of a phospholipase gene from vibrio mimicus**. *Biochim Biophys Acta* 1998, **1394**(1):85-89.
112. Almagro-Moreno S, Boyd EF: **Insights into the evolution of sialic acid catabolism among bacteria**. *BMC Evol Biol* 2009, **9**:118.
113. Vimr ER, Kalivoda KA, Deszo EL, Steenbergen SM: **Diversity of Microbial Sialic Acid Metabolism**. *Microbiology and Molecular Biology Reviews* 2004, **68**(1):132-153.
114. Almagro-Moreno S, Boyd EF: **Sialic Acid Catabolism Confers a Competitive Advantage to Pathogenic Vibrio cholerae in the Mouse Intestine**. *Infection and Immunity* 2009, **77**(9):3807-3816.
115. Lombardo MJ, Michalski J, Martinez-Wilson H, Morin C, Hilton T, Osorio CG, Nataro JP, Tacket CO, Camilli A, Kaper JB: **An in vivo expression technology screen for Vibrio cholerae genes expressed in human volunteers**. *Proc Natl Acad Sci U S A* 2007, **104**(46):18229-18234.
116. Owens L, Busico-Salcedo N: **Vibrio harveyi: Pretty Problems in Paradise**. *The Biology of Vibrios* 2006:266-280.
117. Occhino DA, Wyckoff EE, Henderson DP, Wrona TJ, Payne SM: **Vibrio cholerae iron transport: haem transport genes are linked to one of two sets of tonB, exbB, exbD genes**. *Mol Microbiol* 1998, **29**(6):1493-1507.
118. Pelicic V: **Type IV pili: e pluribus unum?** *Molecular Microbiology* 2008, **68**(4):827-837.
119. Watnick PI, Fullner KJ, Kolter R: **A role for the mannose-sensitive hemagglutinin in biofilm formation by Vibrio cholerae El Tor**. *J Bacteriol* 1999, **181**(11):3606-3609.
120. Shime-Hattori A, Iida T, Arita M, Park KS, Kodama T, Honda T: **Two type IV pili of Vibrio parahaemolyticus play different roles in biofilm formation**. *FEMS Microbiol Lett* 2006, **264**(1):89-97.
121. Marsh JW, Taylor RK: **Genetic and transcriptional analyses of the Vibrio cholerae mannose-sensitive hemagglutinin type 4 pilus gene locus**. *J Bacteriol* 1999, **181**(4):1110-1117.
122. Ariyakumar DS, Nishiguchi MK: **Characterization of two host-specific genes, mannose-sensitive hemagglutinin (mshA) and uridyl phosphate dehydrogenase (UDPDH) that are involved in the Vibrio fischeri–Euprymna tasmanica mutualism**. *FEMS Microbiology Letters* 2009, **299**(1):65-73.

123. Tomich M, Planet PJ, Figurski DH: **The tad locus: postcards from the widespread colonization island**. *Nat Rev Micro* 2007, **5**(5):363-375.
124. Xu C, Wang S, Ren H, Lin X, Wu L, Peng X: **Proteomic analysis on the expression of outer membrane proteins of Vibrio alginolyticus at different sodium concentrations**. *Proteomics* 2005, **5**(12):3142-3152.
125. Xu C, Ren H, Wang S, Peng X: **Proteomic analysis of salt-sensitive outer membrane proteins of Vibrio parahaemolyticus**. *Res Microbiol* 2004, **155**(10):835-842.
126. Mao Z, Yu L, You Z, Wei Y, Liu Y: **Cloning, expression and immunogenicty analysis of five outer membrane proteins of Vibrio parahaemolyticus zj2003**. *Fish Shellfish Immunol* 2007, **23**(3):567-575.
127. Chakrabarti SR, Chaudhuri K, Sen K, Das J: **Porins of *Vibrio cholerae*: purification and characterization of OmpU.** *J Bacterio* 1996(178):524-530.
128. Aeckersberg F, Lupp C, Feliciano B, Ruby EG: **Vibrio fischeri outer membrane protein OmpU plays a role in normal symbiotic colonization**. *J Bacteriol* 2001, **183**(22):6590-6597.
129. Wang SY, Lauritz J, Jass J, Milton DL: **Role for the major outer-membrane protein from Vibrio anguillarum in bile resistance and biofilm formation**. *Microbiology* 2003, **149**(Pt 4):1061-1071.
130. Sperandio V, Giron JA, Silveira WD, Kaper JB: **The OmpU outer membrane protein, a potential adherence factor of Vibrio cholerae**. *Infect Immun* 1995, **63**(11):4433-4438.
131. Lin J, Huang S, Zhang Q: **Outer membrane proteins: key players for bacterial adaptation in host niches**. *Microbes and Infection* 2002, **4**(3):325-331.
132. Inoue T, Matsuzaki S, Tanaka S: **Cloning and sequence analysis of Vibrio parahaemolyticus ompK gene encoding a 26-kDa outer membrane protein, OmpK, that serves as receptor for a broad-host-range vibriophage, KVP40**. *FEMS Microbiol Lett* 1995, **134**(2-3):245-249.
133. Bina JE, Mekalanos JJ: **Vibrio cholerae tolC is required for bile resistance and colonization**. *Infect Immun* 2001, **69**(7):4681-4685.
134. Bailey MJ, Hughes C, Koronakis V: **RfaH and the ops element, components of a novel system controlling bacterial transcription elongation**. *Mol Microbiol* 1997, **26**(5):845-851.
135. Prouty MG, Correa NE, Klose KE: **The novel sigma54- and sigma28-dependent flagellar gene transcription hierarchy of Vibrio cholerae**. *Mol Microbiol* 2001, **39**(6):1595-1609.
136. Kim YK, McCarter LL: **Analysis of the polar flagellar gene system of Vibrio parahaemolyticus**. *J Bacteriol* 2000, **182**(13):3693-3704.
137. McCarter LL: **Polar Flagellar Motility of theVibrionaceae**. *Microbiology and Molecular Biology Reviews* 2001, **65**(3):445-462.
138. D'Autreaux B, Tucker NP, Dixon R, Spiro S: **A non-haem iron centre in the transcription factor NorR senses nitric oxide**. *Nature* 2005, **437**(7059):769-772.

139. Richardson* DJ, Berks BC, Russell DA, Spiro S, Taylor CJ: **Functional, biochemical and genetic diversity of prokaryotic nitrate reductases**. *Cellular and Molecular Life Sciences* 2001, **58**(2):165-178.
140. Pratt JT, Ismail AM, Camilli A: **PhoB regulates both environmental and virulence gene expression in Vibrio cholerae**. *Molecular Microbiology* 2010, **77**(6):1595-1605.
141. Rao NN, Torriani A: **Molecular aspects of phosphate transport in Escherichia coli**. *Molecular Microbiology* 1990, **4**(7):1083-1090.
142. Murakami K, Fuse H, Takimura O, Inoue H, Yamaoka Y: **Cloning and characterization of the iutA gene which encodes ferric aerobactin receptor from marine Vibrio species**. *Microbios* 2000, **101**(400):137-146.
143. Tanabe T, Naka A, Aso H, Nakao H, Narimatsu S, Inoue Y, Ono T, Yamamoto S: **A novel aerobactin utilization cluster in Vibrio vulnificus with a gene involved in the transcription regulation of the iutA homologue**. *Microbiol Immunol* 2005, **49**(9):823-834.
144. Mey AR, Wyckoff EE, Kanukurthy V, Fisher CR, Payne SM: **Iron and fur regulation in Vibrio cholerae and the role of fur in virulence**. *Infect Immun* 2005, **73**(12):8167-8178.
145. Meibom KL, Li XB, Nielsen AT, Wu CY, Roseman S, Schoolnik GK: **The Vibrio cholerae chitin utilization program**. *Proc Natl Acad Sci U S A* 2004, **101**(8):2524-2529.
146. Keyhani NO, Roseman S: **Physiological aspects of chitin catabolism in marine bacteria**. *Biochimica et Biophysica Acta* 1999, **1473**:108-122.
147. Hulsmann A, Rosche TM, Kong IS, Hassan HM, Beam DM, Oliver JD: **RpoS-dependent stress response and exoenzyme production in Vibrio vulnificus**. *Appl Environ Microbiol* 2003, **69**(10):6114-6120.
148. Schild S, Tamayo R, Nelson EJ, Qadri F, Calderwood SB, Camilli A: **Genes induced late in infection increase fitness of Vibrio cholerae after release into the environment**. *Cell Host Microbe* 2007, **2**(4):264-277.
149. Lin YH, Miyamoto C, Meighen EA: **Cloning, sequencing, and functional studies of the rpoS gene from Vibrio harveyi**. *Biochem Biophys Res Commun* 2002, **293**(1):456-462.
150. Merrell DS, Camilli A: **The cadA gene of Vibrio cholerae is induced during infection and plays a role in acid tolerance**. *Mol Microbiol* 1999, **34**(4):836-849.
151. Rhee JE, Kim KS, Choi SH: **CadC activates pH-dependent expression of the Vibrio vulnificus cadBA operon at a distance through direct binding to an upstream region**. *J Bacteriol* 2005, **187**(22):7870-7875.
152. Vimont S, Berche P: **NhaA, an Na(+)/H(+) antiporter involved in environmental survival of Vibrio cholerae**. *J Bacteriol* 2000, **182**(10):2937-2944.
153. Kuroda T, Shimamoto T, Inaba K, Tsuda M, Tsuchiya T: **Properties and sequence of the NhaA Na+/H+ antiporter of Vibrio parahaemolyticus**. *J Biochem* 1994, **116**(5):1030-1038.

154. Nozaki K, Kuroda T, Mizushima T, Tsuchiya T: **A new Na+/H+ antiporter, NhaD, of Vibrio parahaemolyticus**. *Biochim Biophys Acta* 1998, **1369**(2):213-220.
155. Resch CT, Winogrodzki JL, Patterson CT, Lind EJ, Quinn MJ, Dibrov P, Häse CC: **The Putative Na+/H+ Antiporter of Vibrio cholerae, Vc-NhaP2, Mediates the Specific K+/H+ Exchange in Vivo**. *Biochemistry* 2010, **49**(11):2520-2528.
156. Wood RR, Arias C: **Evaluation of Global Gene Expression During Cold Shock in the Human Pathogen *Vibrio vulnificus***. *Mar Biotechnol (NY)* 2010.
157. Yoo L: **The Effect of *rpoH* for Heat Shock Gene Expression on Plasmid Transformation**. *Journal of Experimental Microbiology and Immunology* 2010, **14**:108-111.
158. JeevanJyot, Ghosh A: **Induction of heat shock response in Vibrio cholerae**. *Microbiology* 1995, **141 (Pt 9)**:2101-2109.
159. Waters CM, Lu W, Rabinowitz JD, Bassler BL: **Quorum sensing controls biofilm formation in Vibrio cholerae through modulation of cyclic di-GMP levels and repression of vpsT**. *J Bacteriol* 2008, **190**(7):2527-2536.