REVIEW ARTICLE

Ecological distribution of Vibrios and their significance in coastal ecosystem

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Abstract: Coastal ecosystem is important because it bridges ocean and land. The brackish water receiving nutrients originated from land may nourish heterotrophic bacteria including *Vibrio* species, some of which may pose potential hazards to the public, marine lives and migratory birds in the coastal environment. A rich diversity of Vibrios is evident in the coastal and open oceans, but information on their ecophysiological adaptation and survival is still very limited. Their important roles in the geobiochemical cycles of nutrients have not been exolored adequately. In addition, it also been recently discovered that these Vibrios harbor a very rich of plasmids of various sizes with little knowledge on their function to the hosts. This information deserves attention in *Vibrio* ecology and their role in the various ecosystems for a better understanding of their survival and physiological function.
Keywords:*Vibrio* species; pathogenic; pollution; plasmids; ecological role

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1 Introduction

Vibrio is an old bacterial genus that was first described in the 1800s. The genus name, *Vibrio*, was coined by Pacini in 1854 during his study on cholera disease. Numerous researchers after Pacini subsequently conducted extensive investigations on this group of heterotrophic bacteria are indigenous to aquatic environments, such as ocean, river, inter-tidal water and ponds. *Vibrio* species are Gram negative, straight or curved rods or spirals, and motile by means of flagella (Farmer III and Hickman-Brenner, 1992). Similar to *Pseudomonas*, this is a "big" genus consisting of large number of heterogenous species and isolates, at least 60 different species and many more bacterial isolates are described in this group (www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). As the development of molecular techniques, more new species of *Vibrio* are unraveled and published (Thompson et al., 2005).

Over the history, much of the research on *Vibrio* came initially from medicine and clinical microbiology, and was primarily related to cholera disease, the diarrhea symptom caused by *Vibrio cholerae*, one of the most infamous species in this genus. Other notorious species that are of public health importance include *V. minicus*, *V. parahaemolyticus*, *V. hollisae*, and *V. cincinnatiensis*, etc. Selective species, such as *V. anguillarum* and *V. salmonicida*, do not occur in human clinical specimens, but they are fish pathogens posing a serious threat to aquaculture (Farmer III and Hickman-Brenner, 1992). *Vibrio shiloi*, capable of biosynthesizing and secreting an extracellular peptide (toxin P), inhibits the photosynthesis of coral symbiotic algae (zooxanthellae) and is responsible for the coral bleaching in marine ecosystem (Banin et al., 2000). Although some species of *Vibrio* are of human and animal health concerns, others contribute to the new discoveries and technological development in basic microbiology and biochemistry. Bioluminescence was first found in *V. fischei*, a bacterium named after Bernhard Fisher (Farmer III and Hickman-Brenner, 1992). Lux, a marker gene widely applied in environmental microbiology and microbial ecology to label and track a selective microorganism of interest, is cloned from this species of *Vibrio*. Based on the study of bioluminescence in *V. fischei* and *V. harveyi*, Nealson was the first to propose quorum-sensing mechanism that regulates the bioluminescence and bacterial biofilm formation on surfaces (Nealson and Hasting) and Hasting, (Madigan et al., 2003a; Madigan et al., 2003b; Madigan et al., 2003c; Madigan et al., 2003d; Madigan et al., 2003e; Madigan et al., 2003f).

And such gene-regulation mechanism has been confirmed to be ubiquitous among many other terrestrial bacteria as well (Madigan et al., 2003a; Madigan et al., 2003b; Madigan et al., 2003c; Madigan et al., 2003d; Madigan et al., 2003e; Madigan et al., 2003f). Found worldwide, *Vibrio* species

reside primarily in marine and brackish waters as well as contaminated water including drinking water sources.

Nature reserve including the local one are breeding sites and feeding grounds for a high diversity of coastal species, such as shellfish, migratory birds and ground-dwelling benthic animals and infauna (Tsim and Lock, 2002). Mai Po Nature Reserve holds the largest mangrove stands in Hong Kong and is under pollution from discharge of Shenzhen River and Sham Pui River carrying nutrients-rich water into the reserve. Brackish (slightly salt) water bathing the mangroves contains a diverse community of phytoplankton, zooplankton and water-borne microorganisms (Lin, 1999; Wang et al., 2004; Wang et al., 2006; Zhang and Gu, 2009; Zhang et al., 2006; Zhang et al., 2007; Zhang et al., 2012). Though a number of studies on the ecology of Mai Po Nature Reserve have been conducted in the past two to three decades, little research has ever been carried out to investigate bacterial community, let alone *Vibrio* species. Therefore, the objectives of this study were to isolate and characterize *Vibrio* species from Mai Po Nature Reserve and to establish relationship between unique characteristics and the molecular basis. This literature review will cover the following topics: 1) introduction of Mai Po Nature Reserve; 2) *Vibrio* species and their ecology; 3) antibiotic resistance and plasmid profile in *Vibrio* species.

2 *Vibrio* Species and Their Ecology

2.1 *Vibrio* Species

Cholera is a disease immediately associated with public health and environmental quality; a new branch of public health microbiology was established through the initial investigation by John Snow in London. The causative agent of cholera is *Vibrio cholerae*, one of the most well known species in *Vibrio*. As a genus, these facultative bacteria, belonging to *Vibrio* species, are small comma-shaped rods that live in a wide range of aquatic environment or inside human and animal intestines (Lowrie and Borneman, 1999). *Vibrio* species are members of the family *Vibrio* nacae, which also contains other three genera including *Aeromonas*, *Plesiomonas* and *Photobacterium* with similar characteristics. The bacteria of these four genera are all Gram negative, calatase and oxidase positive, but they can be differentiated by DNA-DNA hybridization and also other distinctive characteristics. For example, DNA-DNA hybridization shows that *Plesiomonas* shigelloides and *Aeromonas* do not have any close relativeness to *Vibrio* or *Photobacterium*. *Aeromonas* genus has a G+C content of 57-63%, which is high in the *Vibrio*nacae family. *Plesiomonas* species fail to grow on the highly selective Thiosulphate-Citrate-Bile salts-Sucrose (TCBS) agar plates, while this selective medium is specially designed for the selective isolation of *Vibrio* species from environmental samples. In addition, vibriostatic agent, O/129 (2,4-diamino-6,7-diisopropylpteridinephosphate) can inhibit the growth of *Vibrio* species, but has no effect on *Aeromonas*. The sheathed polar flagella in *Vibrio* species are important to their movement and bacterial pathogenesis, but all the other three genera do not have such intricate structure in their cells (Collee et al., 1989; Farmer III, 1992.).

Some biochemical characteristics of *Vibrio* species can also be used to differentiate them from other close families in theγ-group microorganisms. *Vibrio* species can be separated from Enterobacteriaceae by a simple oxidase test. Due to the absence of oxidase in Enterobacteriaceae, they do not turn purple on the testing filter paper, showing a negative signal. The facultative *Vibrio* species can grow under both aerobic and anaerobic conditions. In contrast, *Pseudomonas* species only grow in the presence of oxygen as their electron acceptor. Salt requirement is crucial for some species of *Vibrio*, while available Na^+ is not required for the growth of Enterobacteriaceae and Pseudomonadaceae (Farmer III, 1992.; Lowrie and Borneman, 1999).

Due to their wide distribution in seawater, *Vibrio* species have been found worldwide, mainly in marine and brackish water such as coastal waters and estuaries as well as drinking water. Most often, they prefer aquatic environments with rich nutrients and warm temperature. A polluted, relatively physical undisturbed habitat with pH of 8.0 to 9.0 can be optimal for *Vibrio* survival and proliferation (Lowrie and Borneman, 1999). Most *Vibrio* species grow well in the salinities ranged from 5 to 30‰. There are four major factors that govern their distribution: the selective animals or plant hosts, temperature, salinity, and depth below the surface for the species that are found in the ocean (Simidu et al., 1985). The incidence and density of pathogenic *Vibrio* species decrease significantly as water temperatures fall below 20°C (221). The pathogenic *V. cholerae* serogroup O1 is obviously host-adapted and is limited to humans as its host where it causes the disease cholera (Farmer III, 1992.). Other environmental isolates of *Vibrio* species are also found in water column, surface sediment or in association with mollusks and crustaceans (Said and Drasar, 1996). Those species common to open ocean or low nutrient water tend to be smaller and coccoid in shape. It has been reported that multiple-nutrient starvation resulted in a decrease of cell volume by as much as 85% in *V. cholerae* (Baker et al., 1983). The reduced cell size will increase the surface-to-volume ration thereby aiding cells in sequestering more nutrients under the oligotrophic environment. And the smaller cell size will also enhance the bacterial survival by protecting against predation (Byrd, 2000). In fact, the morphological change in cell size is a basic strategy for *Vibrio* species to survive under the starvation condition and stress of natural ecosystem. By living symbiotically with phytoplankton and zooplankton, *Vibrio* species gain a better chance to survive under the low temperature condition and to avoid predation by protozoa. Sometimes, sediment can also serve as reservoir for them by providing organic nutrients and shelter from coldness in winter season (Tamplin et al., 1990).

Much work has been conducted to isolate *Vibrio* species from natural environment, marine animals, plants and the intestinal tract of marine vertebrates. Some species in this group are pathogenic to commercially important fish, oyster and crabs. Some are toxic to higher animals like birds and humans. In the following section, several representatives of *Vibrio* species will be discussed.

2.2 Non-pathogenic *Vibrio* Species

Except for those *Vibrio* species that occur in human clinical specimens, there are about 20 marine species without obvious evidence of any disease association. Theoretically, these marine vibrios could occur in animal feces and pose potential health threat following ingestion of environmental water or not-well cooked seafood. Although some of the species are not proven human-pathogens, they are bacterial pathogens to many animals such as fish, oyster, crab, shrimp and even coral (Farmer III and Hickman-Brenner, 1992).

Vibrio anguillarum is a marine *vibrio* species that causes disease in marine fish and other marine animals. It is a major hazard to fish farms resulting in great economic loss. The infection by *V. anguillarum* to oysters and shellfish inhibits larval swimming to loss of digestive function in adult shellfish. Poor water quality, over-crowding in aquaculture facilities, heavy nutrient loading and high temperature (above 15°C) all contribute to the increasing occurrence of *V. anguillarum* and subsequent outbreak of *vibrio* infection in aquculture (Farmer III and Hickman-Brenner, 1992; Lowrie and Borneman, 1999).

Vibrio alginolyticus is pathogenic to a wide range of marine life including fish, mollusks, crustaceans and cnidarians. It can be found in open ocean, estuaries, sediments, marine life, coral and freshwater, indicating its strong adaptive capability to survive in various aquatic environments. Through exposure to marine water, wounds may be the point of entry for infection by *V. alginolyticus*. As a halophilic marine bacterium, it only grows in the presence of NaCl at concentration and can be as high as 10% (Tison, 1999).

Vibrio fischeri is a halophilic bioluminescent *Vibrio* species. Under the optimal growth conditions, it produces detectable fluorescence. Colonized in specialized organs in fish or squid, this bacterium is of vital importance to the attraction of prey and camouflage in its host animals living in dark deep-ocean. The sites of common occurrence of *V. fischeri* are open ocean, estuaries, fish and crustaceans. In some cases, it may cause gut and organ distention and swelling in the fish. Temperature of 37°C can slower down the bacterial growth while 30°C is optimal (Farmer III and Hickman-Brenner, 1992; Lowrie and Borneman, 1999).

Another halophilic bioluminescent *vibrio* is *Vibrio harveyi*. Some strains of this bacterium are bioluminescent, other are not. Density-dependent expression of luminescence in *V. harveyi* is regulated by the concentration of extracellular signal molecules (autoinducers) in the culture medium and a multiple signaling system of regulating the bioluminescence has been proposed (Bassler et al., 1994). Its virulence to an expanding list of marine animals has been associated with the extracellular products and a toxic cysteine protease. The cysteine protease secreted by *V. harveyi* causes co-agglutination in prawn plasma which leads to uncoverable hemolymph. This event significantly contributes to the pathogenicity of this bacterium in the prawns under farming conditions (Lee et al., 1999).

Vibrio shiloi is believed to be the causative agent of coral bleaching in the Mediterranean Sea area. The β-galactose receptor aids *V. shiloi* to recognize and adheres to the coral surface followed by bacterial penetration to coral tissue. Then, the bacteria multiply in the coral tissue and even enter non-culturable state. In addition, the bacteria can produce heat-stable extracellular toxin to inhibit the photosynthesis of zooxanthellae and heat-sensitive toxin to bleach and lyse algal cells (Banin et al., 2000). It is quite interesting that coral bleaching was affected greatly by temperature. Both laboratory aquarium experiment and natural bleaching shows that a rapid bleaching occurs at about 30°C and no bleaching was noticeable at 16°C. It is proposed that temperature-regulated factors may play a role in bacterial virulence in the bleaching process to coral (Kushmaro et al., 1998).

Due to the length constrains, not every *Vibrio* species can be introduced or discussed here. What has been mentioned above are only representative species. It must be pointed that though some *Vibrio* species are a potential danger to marine life in aquaria and natural aquatic environment, they should not be treated with shear fear but thorough understanding to minimize the potential threat. More work remains to be done to elucidate their role and the presence in the natural environment.

2.3 Pathogenic *Vibrio* Species

Ranking high as major human public health threats, some of *Vibrio* species cause a number of primary enteric infections. Human disease may result from ingestion of water contained with pathogenic *Vibrio* species, consumption of infected seafood, or exposure of wounds to water where active pathogenic *Vibrio* species are present (Lowrie and Borneman, 1999). Among all the *Vibrio* species, there are at least twelve species of clinical significance. They are listed below in the order of medical importance: *V. choleare* O1, *V. cholerae* O139, *V. cholerae* non-O1, *V. parahaemolyticus*, *V. vulnificus*, *V. hollisae*, *V. alginolyticus*, *V. minicus*, *V. damsela*, *V. fluvialis*, *V. metschnikovii*, *V. furnissii*, *V. cincinnatiensis* and *V. carchariae*. The diseases associated with these pathogenic *Vibrio* species comprise cholera, gastroenteritis, bacteremia, wound infections and

meningitis. And the major clinical sources from which these species are isolated are feces, blood, wound and ear (Tison, 1999). The polar or lateral flagella not only facilitate the bacterial motility in natural environment but also aid in attachment of *Vibrio* species to substrates. The pathogenic *Vibrio* species also produce various toxins such as hemolysins, exotoxins and enterotoxins as part of bacterial pathogenesis mechanisms (Lowrie and Borneman, 1999).

The following section will cover the introduction of three most life-threatening *Vibrio* species: *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*. Their characteristics, pathogenesis and ecology are to be introduced below.

2.3.1 *Vibrio parahaemolyticus*

Vibrio parahaemolyticus was first isolated and identified as a causative agent of human disease after an outbreak of acute gastroenteritis in Japan in 1950. A food poisoning due to the consumption of contaminated seafood claimed 20 deaths and 272 cases of acute gastroenteritis in Osaka, Japan. Subsequent extensive investigations revealed an organism to be the etiological agent, which was later named and reclassified as *V. parahaemolyticus* in 1955 (Farmer III and Hickman-Brenner, 1992). Consumption of raw seafood such as oyster and shellfish is a main transmission route for disease development worldwide. Wound, eye and ear infections may also occasionally result from exposure to the marine water where the bacterium is present at relative high population density. The common characteristics of *V. parahaemolyticus* gastroenteritis include nausea, vomiting, abdominal cramps, low-grade fever and chills. The diarrhea is watery and occasionally bloody (Tison, 1999). Ninety-six percent of gastroenteritis–causing *V. parahaemolyticus* are positive for Kanagawa Test meaning that the strain can produce a hemolysin for human red cells. But, only 1% of environmental isolates are positive for Kanagawa test (Farmer III and Hickman-Brenner, 1992). Rehydration is sufficient and effective for the treatment but in case of severe diarrhea, hospital admission is necessary.

The *V. parahaemolytics* strains positive for Kanagawa Test produce an enterotoxin called thermostable direct hemolysin (TDH), a major virulence factor responsible for bacterial pathogenesis. This hemolysin cannot be inactivated by heating at 100°C for 10 minutes and the hemolytic activity is not enhanced by the addition of lecithin indicating a direct action on erythrocytes (Nishibuchi and Kaper, 1995). Acting as a pore-forming toxin, TDH plays an important role in altering the ion flux in intestinal cells thereby leading to a secretory response and diarrhea (Honda et al., 1992; Huntley et al., 1993). Another virulence factor, the TDH-related hemolysin (*TRH*) is generally associated with the Kanagawa Test negative strains or with urease positive strains of *V. parahaemolyticus*. Its *trh* gene may be part of a pathogenicity island. Both tdh and *trh* genes have been widely used as DNA probes and PCR amplification targets in the determination of pathogenic *V. parahaemolytic* of various sources (DePaola et al., 2003).

Morphological observation reveals that *V. parahaemolyticus* is a short Gram negative rod and actively motile in liquid cultures. It is halophilic and fails to grow in peptone water without any NaCl supplement (Collee et al., 1989). It can grow well on TCBS agar plates as greenish-blue colonies, two to three millimeter in diameter. Susceptible to vibriostatic agent O/129, *V. parahaemolyticus*, however, displays a relatively resistance compared to *V. cholerae* (Collee et al., 1989)

As a common marine bacterium, *V. parahaemolyticus* is frequently isolated from sediments, seawater and crustaceans. And its main habitat is probably marine animals with human infection being a second development(Madigan et al., 2003a; Madigan et al., 2003b; Madigan et al., 2003c; Madigan et al., 2003d; Madigan et al., 2003e; Madigan et al., 2003f). It has been isolated from marine and estuarine habitats almost worldwide and despite its halophilic nature. Winter survival of this bacterium has been found to be associated to other marine organisms, copepods in particular. Growth rates are directly related to temperature and the bacterium is rarely found when water temperatures are below 15ºC (Joseph et al., 1982). The bacteria may survival at freezing conditions in shellfish and seafood though at a much lower frequency (Oregon Department of Human Services, 2003). In summer, however, more cases of *V. parahaemolyticys* infection tend to be reported. High temperature at 37 to 42ºC can activate specific heat-shocking proteins and adaptive acid tolerance proteins in the pathogen so that it can stand very acidic environment. Interestingly, the bile-acid-containing environment found in human host favors the growth of virulent strains of *V. parahaemolyticus* and even enhances the expression of virulent factors (Huq et al., 2000).

2.3.2 *Vibrio vulnificus*

Next to the infamous *Vibrio cholerae*, *Vibrio vulnificus* causes the most severe disease. It was originally described by Hoillis as a salt-required organism, distinct from other species of *Vibrio* by its ability to ferment lactose (Hollis et al., 1976). Once named as *Beneckea* vulnifica, it is now universally accepted as *V. vulnificus* (Farmer III and Hickman-Brenner, 1992). Raw oyster consumption by humans is the predominant transmission vehicle of this bacterium. This is quite different from other *Vibrio* species whose transmission vectors cover mollusks and crustaceans as well (Tison, 1999). The septicemia and wound infection caused by *V. vulnificus* progress so rapidly that some are fatal. This bacterium is especially dangerous to people with pre-existing hepatic disease. The increased iron availability due to the liver disease put these individuals at a high risk of being attacked by *V. vulnificus* (Tison, 1999). Gastroenteritis, septicemia, meningitis, pneumonia and keratitis are various diseases caused by this life-threatening pathogen (Farmer III

and Hickman-Brenner, 1992). Additionally, *V. vulnificus* is responsible for skin lesions and necrosis in eels and other susceptible fish all over the world (Lowrie and Borneman, 1999).

Due to its fatality caused to both humans and marine life, *V. vulnificus* has become a research focus for many years. There are considerable variations among the environmental isolates of this bacterium. Sensitive virulence assay with animal model has demonstrated that there are both virulent and avirulent strains (Stelma et al., 1992). The experiment attempting to correlate a particular factor to virulence has proven largely unsuccessful, suggesting that multiple factors contribute to the bacterial pathogenesis (Strom and Paranjpye, 2000). The first virulence factor proven to correlate positively to virulent strain is polysaccharide capsule (PC) surrounding the bacterial cells. Mutations in any gene that adversely affect PC biosynthesis result in a measurable decrease in virulence by *V. vulnificus* (Zuppardo and Siebeling, 1998). Pili, also called fimbriae, mediate the initial attachment and colonization of *V. vulnificus* to its host. The Type IV pili in this bacterium have been isolated and proven to display a comparative similarity to those in *V. cholerae* and other pathogens (Strom and Paranjpye, 2000). Secretion of toxins and enzymes such as chitinases are other mechanisms that facilitate *V. vulnificus* to colonize and adhere to the zooplankton and molluscan shellfish (Strom and Paranjpye, 2000). Cytolysin, one of the most studied hemolysins, is a heat-resistant lytic enzyme that lyses mammalian erythrocytes. This toxin can activates guanylate cyclase to increase the concentration of intracellular cyclic GMP levels, resulting in vasodilation (Kook, 1999). *V. vulnificus* also produces an extracellular protease that has elastolytic and collagenolytic activity. It is designated as metalloprotease. In its pure form, this protein induces hemmorhagic damage and enhances vascular permeability, suggesting its potential virulence in skin lesions (Strom and Paranjpye, 2000). A successful recruitment of enough iron from iron-transporting proteins is crucial for the virulence of *V. vulnificus*. Therefore, the production of hydroxymate and phenolate siderophores ensures the sequestration of iron from host transferrin and hemoglobin. Failure to produce significant siderophores has been associated with reduced virulence in this bacterial pathogen (Simpson and Oliver, 1983).

The sensitive Random Amplification of Polymorphic DNA (RAPD)-PCR technique was recently applied to compare clinical and environmental isolates of *V. vulnificus*. A unique 178 to 200–base pair segment is presented in all clinical isolates but in only a small proportion of environmental isolates. And this unique segment may be used as DNA probe for pathogenic *V. vulnificus* (Warner and Oliver, 1999). Typing of bacteriophage specific to clinical *V. vulnificus* is another method to differentiate environmental isolates from clinical ones. Some virulence factors may originate from certain bacteriophage (DePaola et al., 1998).

V. vulnificus is phenotypically similar to *V. parahaemolyticus*. Most strains of *V. vulnificus* form green, non-sucrose fermentative colonies on TCBS agar plates. The ability of fermenting lactose is a distinct feature to differentiate *V. vulnificus* from other *Vibrio* species. The absence of NaCl addition or high concentration of salt ($\geqq$8%) can curb the bacterial growth in the nutrient broth completely. Very sensitive to vibriostatic agent, *V. vulnificus* does not grow in the presence of 10 µg/ml of O/129 (Farmer III and Hickman-Brenner, 1992).

V. vulnificus is a naturally occurring, free-living inhabitant of estuarine and marine environment. And its presence in estuarine environment may not be related to pollution or other forms of contamination (Høi et al., 1998). Preferring to warm climates, it has been isolated mostly from waters where temperatures range from 10 to 30°C. When the temperature exceeds 18°C, it can multiply quite well (Kaspar and Tamplin. 1993) However, if temperature is less than 10°C, the total viable count drops greatly, almost to undetectable level (Strom and Paranjpye, 2000). Temperature is proposed to be an important factor inducing the bacterial entry to VBNC state. *V. vulnificus*, incubated at 5°C, enters into VBNC state no matter it was in nutrient-deplete artificial seawater or nutrient broth (Oliver, 2000). Physiological age of bacterial cells seems to be another factor that affects the time required for entry to VBNC state. Cells in stationary phase took twice the time to become nonculturable at 5°C than did logarithmic-phase cells (Oliver et al., 1991). Transferred from normal temperature (22°C) to low temperature (4°C), there was an observable decrease in biosynthesis of macromolecules in *V. vulnificus*, suggesting signal transduction occurred in response to down-shift of temperature. Along with an immediate decline in protein synthesis, some "cold shock" proteins were also induced to ensure survival in cold environment (Oliver, 2000). Morphological changes in cell size along with alteration in membrane fatty acid profile can also be noticed after bacterial entry to VBNC state (Strom and Paranjpye, 2000). However, the potential virulence of *V. vulnificus* in VBNC state can never be overlooked. Even a small dosage of VBNC cells of this bacterium is sufficient to kill mice (Oliver, 1993).

Generally speaking, low to moderate salinity favors the growth of *V. vulnificus*, whose normal salinity range falls between 1.5 to 2.5% (Strom and Paranjpye, 2000). With its chitinase activity, *V. vulnificus* may associate with zooplankton or other filter-feeding mollusks such as oysters, clams and mussels. The bacterium can be accumulated and reside in animal gut or other tissues. Its concentration in fish intestines is also well-documented and fish may serve as a reservoir for the transport of the bacterium (Motes et al., 1998). The association of *V. vulnificus* with zooplankton and phytoplankton or its attachment to soft submerged sediments is an effective tactics to survive in stressful conditions like coldness and starvation (Strom and Paranjpye, 2000).

2.3.3 *Vibrio cholerae*

Vibrio cholerae is by far the best understood species in the genus of *Vibrio*. As the causative agent of cholera, it has claimed millions of deaths and become one of the most feared bacterial pathogens in history. This organism was first described by Pancini in 1854, the same year John Snow investigated the link between drinking water quality and cholera in London. Then Robert Koch confirmed its bacterial etiology in 1883 when he successfully isolated the cholera bacillus from pond water during a cholera outbreak in Egypt (Said and Drasar, 1996). Classified by serological testing for O antigen determinants, *V. cholerae* can be subdivided into three major groups, *V. cholerae* O1, *V. cholerae* non-O1 and *V. cholerae* O139 Bengal. And *V. cholerae* O1 can be further categorized into three subtypes: Inaba, Ogawa and Hikojima, based on the agglutination with specific antisera. The bio-grouping of *V. cholerae* O1 into Classical and El Tor is also widely used, when mentioning the etiologic agent of epidemic cholera (Said and Drasar, 1996). Cholera was often documented in early writings as Asiatic cholera due to its epidemic range in India. However, since 1800, there have been seven severe pandemics of cholera recorded over the medical history. The first pandemic began in 1816–1817 and six other pandemics were reported with a beginning in 1829, 1852, 1863, 1881, 1889, and 1961 respectively. Although the seventh pandemic in 1961 was caused by the El Tor, the Classical biogroup, responsible for the previous six pandemics, has displaced the El Tor in some parts of the Indian subcontinent (Farmer III and Hickman-Brenner, 1992). The general belief that cholera is only limited to *V. cholerae* O1 was challenged in October, 1992. An epidemic of cholera outbreak in Madras, India was confirmed to be caused by a newly-emerged *V. cholerae* serogroup, designated as O 139 Bengal. This group of *V. cholerae* appeared to spread very rapidly from India to United Kingdom to United States, accountable for a wide-sweeping of cholera outbreak in 1994 and 1996 (Tison, 1999).

In the area of cholera epidemic, individuals who ingested *V. cholerae* may have either mild diarrhea or very acute symptoms. In extreme cases, patients will develop an acute diarrhea with constant purging that has been called "cholera gravis." Vomiting and no desire to eat are also commonly accompanied symptoms. If left untreated, the patient will encounter more severe dehydration, electrolyte imbalance, painful muscle cramps, watery eyes, loss of skin elasticity and anuria. The death from dehydration is usually imminent after onset of above-mentioned symptoms (Farmer III and Hickman-Brenner, 1992). Most often, the immediate medical treatment and management can prevent the fatality of patient with cholera. The widely practiced management includes: 1) rapid replacement of water and salt(s); 2) maintenance of normal hydration; 3) reduction of both magnitude and duration of diarrhea; and 4) prompt introduction of normal diet to minimize the nutrient loss (Cook, 1996).

Other serogroups besides O1/ O139 have been collectively referred to *V. cholera* non-O1. Though they do not produce cholera toxin nor cause cholera epidemic, they are etiologic agents of self-limiting gastroenteritis and some may cause wound-infections and bacteremia (Tison, 1999). In this large and diverse species, there are still other harmless aquatic strains. Lack of toxin-producing genetic elements in their genome, non-pathogenic *V. cholerae* non-O1 live freely in natural environment. But due to similarity to the pathogenic counterparts, they have a potential to "up-take" pathogenicity islands and convert to toxic strains (Karaolis et al., 1998).

The efforts to search for an effective cholera vaccine have mainly focused on the bacterial pathogenesis or virulence factors of *V. cholerae*. It turns out that *V. cholerae* is an excellent model for studying bacterial colonization and effects of toxin on host cells (Levine and Kaper, 1996). The bacterial infection commences with ingestion of seafood or drinking water contaminated with pathogenic *V. cholerae*. If the bacterium successfully penetrates the acid barrier of the stomach through various mechanisms such as acid tolerance response, it may pass through the pylorus and initiate a series of colonization and infective reactions (Levine and Kaper, 1996). The single flagellum may contribute to its virulence by aiding the bacterium to arrive at mucosa. There is evidence that flagellum-free mutant strains are much less virulent than their motile parental strains (Salyers and Whitt, 2002). The filamentous pili that form bundles at one end of *V. cholerae* are designated as Tcp pili (toxin coregulated pili) for the genes encoding pili are regulated similarly to genes encoding cholera toxin. Among the 15 genes encoding Tcp pili, 14 are clustered on what is now called a pathogenicity island (Karaolis et al., 1998). The accessory colonization factor (acf) genes are also located on this pathogenicity island, which have been suggested encoding an adhesin. Some research also indicated that Tcp pili may serve as a receptor for the bacteriophage that introduced the subunits of cholera toxin. Therefore, Tcp pili play an important part in the virulence evolution in pathogenic *V. cholerae* (Salyers and Whitt, 2002). The virulence of cholera toxin (CTX) has never been questioned since its discovery. Naturally occurring strains or ctx-free mutants of *V. cholerae* that do not produce CTX could not cause cholera in human volunteers. Due to the presence of other toxins, such strains may however cause milder diarrhea, cholera toxin is an A-B ADP-ribosylating toxin containing one A (enzymatic) subunit and B (binding) subunit (Levine and Kaper, 1996). Simply, CTX toxin catalyses the transfer of the ADP-ribose moiety of NAD to a specific arginine residue in the Gsαprotein, resulting in the activation of adenylate cyclase and subsequent increase in intracellular levels of cAMP. Then cAMP activates a cAMP-dependent protein kinase, leading to protein phosphorylation, alteration of ion transport and ultimately severe diarrhea (Levine and Kaper, 1996). Recent study on the genome sequence of *V. cholerae* concludes that ctx genes actually came from a virulent filamentous phage CTX (Karaolis et al.,

1998). In addition to notorious CTX toxin, *V. cholerae* may produce other enterotoxins. ZOT toxin (zonula occludens toxin) is one of them. ZOT toxin affects the structure of the tight junctions, allow contents of the lumen to diffuse into underlying tissue and disrupt the ion balance and thereby cause diarrhea. And zot gene has been found to lie immediately upstream of ctxA/B operon on the CTX phage (Salyers and Whitt, 2002). ACE toxin (accessory cholera enterotoxin) is a newly discovered toxin, whose role in human disease is still unclear. But it does cause fluid accumulation in rabbit ideal loop model, which is a trait for many enterotoxins. Interestingly enough, ace gene is also located closely linked to zot (Karaolis et al., 1998; Salyers and Whitt, 2002). It seems that almost all major virulence genes are located close to each other on a CTX phage, which has been "raptured" and integrated into *V. cholerae* genome. Besides all the major toxins mentioned, there are other toxins that have been identified: haemolysin/cytolysin, shiga-like toxin, heat-stable enterotoxin, new cholera toxin and sodium-channel inhibitor, to name a few (Levine and Kaper, 1996).

V. cholerae is the type species of genus *Vibrio*. It is a small short or curved rod, 1.4 to 2.6 μm in length. Distinct from other halophilic vibrios, *V. cholerae* grows in a complex medium without added salt, though its growth only reaches 50 to 80% of optimal level. High salinity of 8% NaCl in the medium will inhibit its growth completely. Preferring alkaline conditions, this bacterium can grow quite well in conditions where pH is as high as 10 (Bradford et al., 1994). Overnight grown colonies of *V. cholerae* on TCBS agar plates are generally yellow due to sucrose fermentation, a characteristic different from the closest cousin, *V. minicus* (Farmer III and Hickman-Brenner, 1992). The colonies are typically big (3-4 mm in diameter), smooth, circular, glistering and a little bit fattened (Farmer III and Hickman-Brenner, 1992). Visible growth at 42°C is recorded though its optimal growth temperature is 35 to 37°C. However, *V. cholerae* can be killed by heat at 60°C for 10 minutes. This is the reason that thorough cooking is a simple and sufficient method to eradicate *V. cholerae* infection. Low temperature at 4°C is often used as an inducer for its entry to VBNC state. Very sensitive to vibriostatic agent, the bacterial growth can be stopped in the presence of 10 μg/ml of O/129 (Sakazaki, 1989).

Historically, *V. cholerae* was divided into subgroups and there was controversy as to the classification of *V. cholerae* O1 and *V. cholerae* non-O1. Later on, 16S rDNA, numerical taxonomy and DNA/DNA hybridization have all supported the hypothesis that *V. cholerae*, both O1 and non-O1, represents a single species. And isolates of environmental and clinical sources are identical in terms of 5S rDNA sequence (Colwell et al., 1989). In the past, it was generally accepted that highly adapted *V. cholerae* O1 is able to exist for only very short period of time outside human intestine. But the accumulated evidence shows that *V. cholerae* is actually an autochthonous inhabitant of brackish water and estuarine system (Colwell et al., 1989). With initiation of a series of genetic and physiological changes, *V. cholerae* displays dynamic adaptations to the changes in the environmental parameters, including low nutrient concentration, pH in the range of 7 to 8, fluctuating temperatures and exposure to UV via sunlight. It may be able to persist in the natural environment or to colonize plants or animals, which facilitates its survival during inter-epidemic periods (Islam et al., 1996). The isolation of *V. cholerae* O1 from foodstuffs such as sewage-contaminated vegetables and fruits has been reported, suggesting that the bacterium can survive in soil and sewage for an extended period of time. Fish and shellfish, incriminated in cholera outbreak, are not only contaminated with *V. cholerae* on the surface but also in their intestinal tracts or guts, both of which may act as reservoirs for the bacteria (Colwell et al., 1989; Madigan et al., 2003a; Madigan et al., 2003b; Madigan et al., 2003c; Madigan et al., 2003d; Madigan et al., 2003e; Madigan et al., 2003f).

Among the various environmental parameters that are important determinants of ecology of *V. cholerae*, salinity and temperature relationships are the first factor that deserves special attention. A nice correlation between *V. cholerae* isolated from natural environment and salinity level was established, with greater frequency of isolations at sites of salinities between 0.2 to 2%. The effect of temperature was more obvious: isolations were more frequent and readily obtained when the water temperature was greater than 17°C (Colwell et al., 1981). This finding was also supported by Hood and his co-workers, who reported that a calculated salinity of 1.8% and temperatures between 20 and 35°C were optimal for recovering *V. cholerae* from water, oyster, blue crabs and sediment samples in Florida (Hood et al., 1983). The constantly changing conditions in tidal estuaries suggest that *V. cholerae* may adapt to a wide range of saline and temperature conditions in natural habitats.

The ability to associate with specific surfaces in vicinity also exerts some influence on survival chances in the natural environment. Adherence to chitin, the principal component of crustacean shells, is of great interests to many researchers. Some experiments have proven that chitin-absorbed *V. cholerae* was able to survive the very acidic conditions suggesting that such adherent organism may pass through the gastric acid barrier in human intestine (Colwell et al., 1989). In the natural environment where nutrients are depleted, *V. cholerae* tends to adhere to inert particles over chitinaceous surface to seek an ecological advantage. For instance, the attachment can mediate a transport of *V. cholerae* to sediment where it has a better chance to contact with organic particles than in water column (Hood and Ness, 1982). Apart from using chitinaceous particle as a vehicle, *V. cholerae* can utilize chitin as nutrients in time of food-depletion (Islam et al., 1996). The attachment to copepods is a well-recorded phenomenon. (Huq, 1981) studied the role of copepods on

survival and multiplication of *V. cholerae* in microcosms and found that the organism survived longer and multiplied in the presence of copepods (Colwell et al., 1981). His research was further supported by Amako (1987) who observed that at low temperature (0°C) *V. cholerae* attached to copepods survived more than one week, compared with less than one day without copepods (Islam et al., 1996).

Aquatic flora can also serve as reservoirs for *V. cholerae*. The marine algae produce a number of extracellular products which contain peptides, amides and nitrogen which explains why the amino-acid requiring bacteria, including *V. cholerae* are predominant on algal surfaces. With the secretive products from algae, the *V. cholerae* would be able to persist at least several months necessary to span the inter-epidemic period (Islam et al., 1996). The peak incidence of cholera in endemic areas of Bangladesh occurs together with the bloom of the blue-green algae in the natural aquatic environments, which is also an evidence to support the above findings (Islam, 1994; Islam and Bateman, 1994).

The most profound challenge to concepts concerning *V. cholerae* ecology comes from Colwell's studies, which proposed that it possesses the ability to enter a state of dormancy in response to nutrient deprivation, elevated salinity and/or reduced temperature (Colwell et al., 1994). Under the oligotrophic environments, *V. cholerae* produces its progeny cells significantly decreased in cellular size. Morphology also alters from typical bacillus to a coccoid shape with reduction of macro-molecules biosynthesis (Gauthier, 2000). As far as osmolarity is concerned, it should be noted that osmotic up-shift drastically inhibits the transport of carbohydrates and amino acids and thus may induce VBNC response to the bacterium through nutrient starvation (Roszak and Colwell, 1987). And again, living organisms have a protective effect on microorganisms in various environments. The association of *V. cholerae* to zooplankton (mainly copepods) in seawater has been proven to lower their entry to VBNC state and protect them against Al and chlorine treatments (Chowdhury et al., 1997).

A variety of environmental parameters may induce or favor the nonculturable responses in *V. cholerae*. In compliance with the universal law of simplicity and economy, *V. cholerae* in natural environments uses the VBNC responses to cope with encountered multistress. However, the public health significance of VBNC *V. cholerae* should never be overlooked. Cells of *V. cholerae* in VBNC state may still be capable of causing human cholera (Oliver, 2000). This finding supported the hypothesis that cells of VBNC *V. cholerae* are able to resuscitate to culturable and infectious state by following human passage. The PCR amplification of the CTX operon from VBNC cells of *V. cholerae* also enforces the concept that failure to culture *V. cholerae* from environmental or clinical samples does not prove the absolute absence of the bacterium (Huq et al., 2000). Therefore, the extensive studies in the past decades are sufficient to conclude that VBNC *V. cholerae* cannot be readily considered "dead" but rather need to be viewed as a potential public health threat.

2.4 The Climate Links with *Vibrio* Diseases

Cholera, one of the most noticeable diseases caused by *V. cholerae* displays a very clear seasonal pattern of epidemics. Cholera epidemic peaked during November to January with a second, small peak during May and June in India. This pattern has been repeated at different cholera epidemic areas in history. It is a pity that no satisfactory explanation has been put forward to explain the pattern of cholera so far (Islam et al., 1996). In addition, as mentioned already, temperature-induced phytoplankton blooms have been found to link with epidemic in certain regions of the world (Islam, 1994; Islam and Bateman, 1994). The climate-linked temperature changes have a remarkable effect on the incidence of vibrios in the natural environment. The climate warming affects the host-pathogen interaction by increasing pathogen development rates and transmissions by relaxing over-wintering restrictions on pathogen life cycle and by modifying host susceptibility to pathogen infections (Harvell et al., 2002). For example, the El Nino-induced weather change is believed to produce higher level of contamination in shellfish, which resulted in 250 cases of *V. parahaemolyticus* infections in humans in Northwest of USA in 1997 (Tamplin, 2001). And the wide presence of *V. vulnificus* in oyster meat and sediments correlates significantly with the periodicity of human *vibrio* infection in Gulf of Mexico (Jackson et al., 1997).

Besides human diseases, a dramatic global increase in the severity of coral bleacing in 1997 to 1998 is coincident with high El Nino temperatures. This coral bleaching is geographically extensive and caused great mortality. The bacterial pathogen of coral bleaching, *V. shiloi*, is temperature sensitive and grows well at the temperatures close to host optima (Kushmaro et al., 1998). *V. fluvialis* and *V. furnissi* can also quickly settle on coral tissue and begin to decompose (Lowrie and Borneman, 1999). Growth rates of marine bacteria are positively correlates with temperature in a certain range. Therefore, the increase in bacterial growth and multiplication due to the warmer temperature will have a more severe threat to coral species. Links between climate change and *vibrio* disease will contribute to hazards of public health and marine life in a profound way. More work still remains to be done to establish appropriate models that elucidate the relationship between *Vibrio* species and climate changes, thereby predicting possible outbreak of diseases such as cholera or coral bleaching.

2.5 *Vibrio* Species and Wildlife

Previous studies proved that *V. cholerae* non-O1 is much easier to isolate than O1 group from the environment and indeed is much more frequently isolated. And non-O1 group is not common from clinical sources (Lewin, 1996). Non-aquatic animals as reservoirs of *V. cholerae* were

reported in early 1980s. A study in UK cultured *V. cholerae* non O1 from 6% of gulls sampled and from two mute swans (Lee et al., 1982). *V. cholerae* non O1 was also isolated from farmed ducks in Denmark (Bisgaard and Kristensen, 1975). Ogg and his co-worked isolated both O1 group and non-O1 group of *V. cholerae* from feces of 20 species of aquatic birds in Colorado. Two of the species, great blue heron and ring-billed gull harbored O1 vibrios. The researchers also suggested that aquatic birds may serve as carriers and disseminate *V. cholera* through droppings over a wide area (Ogg et al., 1989). The harboring of *V. cholerae* in some aquatic birds seems to be a reflection of diet which is predominantly seafood or drinking water contamination.

Bacterial disease in birds, especially poultry can bring great economic losses. *Vibrio* species have been listed as one of the bacterial pathogens in poultry. For instance, *V. cholerae* non-O1 has been isolated from the liver of dead goose, from nasal cavities of apparently healthy ducks and from tissues of ducks with septicemia (Barnes, 1997). *V. mestchnikovii* was isolated and identified to be the causative agents of diseased domesticated ducks, muscovy ducks and geeses in Germany (Hinz et al., 1999). It is also thought to produce sub-acute hemorrhagic enteritis in water fowls (Lowrie and Borneman, 1999). *V. parahaemolytics*, most commonly associated with sediments and zooplankton, can cause enteritis in avian species like myhahs, canaries, parakeets and finches (Lowrie and Borneman, 1999). The devastating high mortalities of birds and fish in Salton Sea, California in 1996 were finally confirmed to be caused by *Vibrio* species infection. Researchers believed that consumption of fish sickened by *V. alginolyticus* was the main infection passage in fish-eating birds (http://www.desertusa.com/salton/salton.html). Another explanation for large die-offs of migratory birds in Salton Sea sounds a little bit “indirect”. The infection of *Vibrio* species in fish lead to lesions, swelling of body cavity and major fatal changes in the internal organs, which might triggered the germination of botulism bacterial spores followed by secretion of toxin in the fish. If the birds consumed those sick fish, it was very likely that birds would be poisoned by deadly dosage of botulism present or infected with botulism bacteria which later caused avian cholera in birds (Kaiser, 1999). The relationship between bird health and *Vibrio* species, either as carriers or victims, calls for more attention and further study, for the sake of public health and bird protection.

3 Antibiotic Resistance and the Plasmid Profile

The rapid spread of antibiotic resistance in bacteria, especially bacterial pathogens, is an increasingly serious problem to our society. High levels of antibiotic resistance in microorganisms in both natural and clinical environment are the direct consequence of indiscriminate use of antibiotic drugs in humans and veterinary medicine (Andersen et al., 1994). Urban effluent which may contain various antibiotic resistant bacteria or a range of antibiotics is discharged into natural environment with no restriction currently. River waters, receiving sewage, are the main receptor for those pollutants. As rivers are the major water sources for human and animal consumption, this pollution may contribute to the maintenance or even spread of bacteria with antibiotic resistance (Goñi-Urriza et al., 2000). Sea is where all the river waters flow to, which is also the final destination of those antibiotic resistant bacteria or antibiotics. Several studies have focused on the incidences of antibiotic resistant bacteria in marine environments like sediments, coastal canal water and ocean water (Andersen et al., 1994). *Vibrio* species, part of natural marine environment, are at no exception to be exposed to antibiotics. Since some species come from medical sources, where antibiotics are most frequently administered, investigation on their antibiotic resistance and mechanisms is of special significance. The extensive study on antibiotic resistance in bacteria has also led to an increasing interest in the research of plasmid-medicated transfer of drug resistance, not only in hospital environment, but also in natural environment (Sandaa and Enger, 1994). Many papers have been published on the antibiotic resistance mediated by Resistance (R) plasmids in marine environment (Sizemore and Colwell, 1977; Baya et al., 1986). Those Gram negative marine bacteria are capable of receiving plasmids by conjugal gene transfer or other means.

Vibrio species are usually susceptible to tetracycline, chloramphenicol, the aminoglycosides and nalidixic acid, but susceptibility to other antibiotics may vary according to species or strains. For instance, many strains of halophilic species including *V. parahaemolyticus*, *V. alginolyticus*, *V. fluvialis*, *V. furnissii*, *V. damsela* and *V. metschinikovii* are resistant to ampicillin and β-lactase production in these species was reported. However, most strains of *V. vulnificus* and *V. hollisae* are susceptible to ampicillin. For these halophilic strains, the resistance is more likely to be intrinsic rather than plasmid-mediated (Sakazaki, 1989). In the past, *V. cholerae* O1 was generally susceptible to tetracycline, the choice of medicine for cholera treatment. As a result of extensive use of tetracycline and chloramphenicol drugs, plasmid-mediated resistance to a wide range of antibiotics in *V. cholerae* O1 has been found in Tanzania and Bangladesh (Sakazaki, 1989). Most of the *V. cholerae* O139 isolates from outbreak in Bangladesh and India in 1992 were recorded to be resistant to trimethoprim-sulfamethoxazole, streptomycin, furazolidone and vibriostatic agent O/129 (Bradford et al., 1994). The corresponding resistance genes were later found to be located on large conjugative elements (SXT constins) that are integrated into *V. cholerae* chromosome (Hochhut et al., 2001).

Among various mobile genetic elements that may contribute to the antibiotic resistance among *Vibrio* species, the conjugative resistance plasmid (R plasmid) received the most attention. The plasmid-encoded tetracycline resistance determinant was detected in *V. salmonicida* isolated from

the fecally polluted sediment (Andersen et al., 1994). The conjugation of a series of multiple antibiotic resistance plasmids of different sizes took place between *V. cholerae* and *Aeromonas salmonicida* in seawater. And the efficiency of transfer was not temperature-dependent for bacteria (Kruse and Sorum, 1994). In Albania and Italy, *V. cholerae* O1 El Tor strains isolated from 1994 outbreak of cholera were characterized by their resistance to tetracycline, streptomycin, trimethoprim, sulfathiazole and O/129. The resistance genes were identified to be on a self-transferable 60 MDa conjugative plasmid (Farmer III, 1992.). In the presence of antibiotics, plasmids may utilize external selective forces for their own maintenance and their spread in *Vibrio* species may be proportional to the intensity of selective forces of antibiotics (Baquero et al., 1997). Under the exposure of antibiotic challenge or fluctuating environment, plasmids encoding a single type of resistance may be lost and they need to evolve to capture antibiotic resistance determinants from bacterial chromosome or other sources. The environment containing multiple antibiotics can lead to plasmid evolution towards acquisition of multiple antibiotic determinants (Baquero et al., 1997).

Besides R plasmids, other mobile genetic elements may also contribute to the antibiotic resistance in *Vibrio* species. Integrons can acquire open reading frames (gene cassettes) and convert them to functional genes. So far, more than 40 kinds of antibiotic resistance cassettes have been characterized in these structures (Koonin et al., 2001). PCR analysis of *V. cholerae* O1 strains of cholera outbreak in Vietnam clearly demonstrated the presence of Class I integrons which harbored a gene cassette encoding resistance to streptomycin and spectinomycin (Dalsgaard et al., 1999). VCRs (*V. cholerae* repeated sequences) have been detected frequently in *V. cholerae* genome and their gene organization is identified to be similar to many well-characterized antibiotic resistance integrons. Confronted with antibiotic challenges, such structures would "capture" resistance genes through integrase mediated process and confer host strain antibiotic resistance. For the purpose of "gene capture", VCRs have been found in many *Vibrio* species, suggesting that this mechanism of heterologous gene acquisition is ubiquitous (Mazel et al., 1998).

Plasmids isolated from marine environment have been shown to be unrelated to numerous plasmid types previously known from clinical and environmental sources (Smalla and Sobecky, 2002). As a potential source of untapped and novel genes with possible biotechnological importance, plasmids from marine bacteria are receiving increasing attention. By means of plasmid mobilization, marine bacterial community can readily obtain plasmid-encoded trait and adapt to changing environmental conditions. The examples of plasmid-conferred advantages are protection from UV light damage, resistance to heavy metals and antibiotics, and catabolism of xenobiotic compounds (Sobecky, 1999). Research on plasmid ecology in marine sediment microbial communities showed that *Vibrio* species harbored different replication sequences, indicating a high diversity among bacterial population in marine sediment (Sobecky et al., 1998). *Vibrio* species were also reported to adapt to the oligotrophic conditions and maintained the ability to transfer plasmids. The results provided evidence that plasmid-mediated gene transfer is likely to be an important factor in determining marine bacterial community structure and function (Goodman et al., 1993). With self-transfer and mobilization features, plasmids are believed to promote the dissemination of advantageous genes throughout the naturally occurring bacterial communities in marine environment. Therefore, the study of plasmid ecology in marine *Vibrio* species is of importance. It contributes to a better understanding of bacterial resistance to antibiotics, heavy metals and toxic organic chemicals in the natural environment.

Environmental isolates of *Vibrio* species isolated from water and sediment of Mai Po Nature Reserve, Hong Kong SAR were strongly resistant to β-lactam family of antibiotics, but susceptible to tetracycline, chloramphenicol, nalidixic acid and streptomycin (Wang et al., 2004; Wang et al., 2006). In addition, *V. cholerae* MP-1 was very tolerant to high concentration of vibriostatic agent O/129 to 40 μg/ml, at which the growth of *V. aestuarianus* MP-2 and *V. vulnificus* MP-2 was completely inhibited. *V. cholerae* MP-1 did not show any apparent growth at 15ºC, but was adapted to a much wider environmental pH from 5.2 to 9.2 for growth while *V. vulnificus* MP-2 was more sensitive to pH changes yielding the highest biomass at pH 6.2 (Wang and Gu, 2005). *A. salmonicida* MP-4 was surprisingly tolerant to salinity as high as 60.0‰ NaCl and grew almost equally well as under conditions of other treatments. *V. vulnificus* MP-4 was the most sensitive to UV and Fe3+ treatments. *A. salmonicida* MP-3 was only inhibited by 100 mM H_2O_2 while the other three strains did not show any growth at 10 mM H_2O_2. A small naturally occurring plasmid was found in *V. cholerae* MP-1 while no plasmid was detected in the other two bacteria (Zhang et al., 2007). This study suggests that Mai Po Nature Reserve harbors bacteria of unique characteristics that warrant further investigation.

4 Nature Wetland

The Mai Po Inner Deep Bay Ramsar site covers approximately 1500 hectares in the northwestern New Territories, Hong Kong Special Administrative Region. Consisted of extensive inter-tidal mudflats, dwarf mangroves, *gei wai*, reedbeds, fishponds and drainage channels, the Mai Po Inner Deep Bay Ramsar site plays an important role in supporting a wide range of wild life including migratory birds and local species (Tsim and Lock, 2002). This area was officially listed as a "Wetland of International Importance" under the Ramsar Convention in 1995. The 380-hectare Mai Po wetlands, covering mainly mangroves and *gei wai*, are the largest mangrove stand in Hong Kong and declared as a

nature reserve with special habitat and visitor management (Tsim and Lock, 2002).

Mangroves are very unique inter-tidal wetland ecosystems along subtropical shores, receiving nutrient inputs from regular tidal flushing, freshwater streams and rivers. Mostly inundated by incoming tides twice a day, Mai Po mangrove habitats are characterized by fluctuating salinity, alternating aerobic and anaerobic conditions, periodic wet and dry environments, and an unstable and shifting substratum (Tam and Wong, 2000). Mangrove plants in Mai Po Nature Reserve are perennial plants belonging to diverse taxonomy but all share similar specialized morphological, structural and physiological adaptations. With salt glands, knee roots, aerial roots and viviparous reproduction, those mangrove plants survive successfully in the harsh inter-tidal environment of salinity stress and mobile substratum (Lee, 2002). Through photosynthesis, mangrove plants can produce large quantities of organic matter and provide food for small ground-dwelling benthic animals by means of leaf litter. Heterotropic bacteria and fungi also contribute to the decomposition of falling leaves and initiate a food chain that sustains other larger animals. It is believed that mangrove plants are a major source of food for marine animals in subtropics and tropics (Lee, 2002). With the abundant food resources and relatively stable water hydrology, Mai Po mangrove acts as a natural nursery to the juveniles and larvae of fishes and prawns that are of economical importance. It provides food based on its own production through photosynthesis and shelters juveniles and larvae from predators and turbid estuarine water. Apart from the protection of fish and prawn juveniles and larvae, a large number of above-ground plant structures make the habitat spatially complex. Close to the sediment surface, all these structures serve as the attachment sites for numerous sedentary animals such as oysters and barnacles. Microscopic diatoms and other unicellular algae are an additional food source to invertebrates in the mangrove. Sea-grasses are another vital component in Mai Po mangrove that support juvenile and larval stage of crustaceans and fishes (Lee, 2002). Mai Po mangrove houses not only a huge number of marine invertebrates of various classes, it is also the home to many reptile species such as snake, lizards and turtles (Tsim and Lock, 2002). As an ecologically rich area, Mai Po mangrove is the paradise for a wide range of flora and fauna.

Besides mangroves, Mai Po Nature Reserve includes other types of habitats such as *gei wai*, fish ponds, reedbeds and inter-tidal mudflats. *Gei wai*, the tidal ponds traditionally operated for aquaculture, covers about 230 hectares in Mai Po. Litter input from pond plants and the organic materials brought through the single sluice gate at high tides are the major nutrient sources. The profuse emergent vegetation provides the best nursery to shrimps and fish stocks. In winter, *gei wai* is drained to harvest the commercial fish and shrimps or to provide foods for hundreds of thousands of migratory birds (Tsim and Lock, 2002). The 470-hectare fish-ponds are the dominant habitats in the Inner Deep Bay Ramsar site. Each year, about 4000 tons of fresh water fish are produced from this area. Reedbeds in Mai Po Nature Reserve is the largest reedbed area in Hong Kong and Guangdong Province in P. R. China. Both reedbeds and fish ponds are valuable habitat for insects. Over 400 species of dragonfly, butterfly and moth have been recorded in or near this area. The Mai Po reedbeds also support a unique bird community. It is the only place in the world where Styan's Grasshopper Walber can be found in winter. The reed stems were used to be collected for making paper and thatching house roofs. The underground rhizomes (similar to roots) can be used as Chinese traditional medicine. As an integrated part of wetland, reedbeds function as water-cleaners by absorbing and metabolizing organic substances (http://www.ecc.org.hk/3efebinfo7.htm). The last prominent habitat in Mai Po Nature Reserve is the inter-tidal mudflat located at the delta of the Shenzhen River. Mudskipper and fiddler crabs are commonly found animals on and in the mudflat. Some crabs play a role in nutrition recycling by consuming leaf litters while others sieve out small animals and algae from mudflat sediments. And mudskippers prey on small invertebrates or microscopic algae in the mudflat. All these organisms are of paramount ecological importance to the biodiversity in Mai Po Nature Reserve (Lee, 2002). Though inter-tidal mudflat is a dynamic environment with changing salinity, its role in providing food and habitats for wildlife, especially for foraging birds, is much greater than previously believed.

Due to the fact that it supports about 49 to 68 thousand water birds in winter and more than 100 thousand birds in the entire year, Mai Po Nature Reserve has become the habitat for conservation of bird species of international and local importance. Globally threatened species such as Black-face Spoonbill, Oriental stork and Normann's Greekshank have all been recorded in Mai Po Nature Reserve (Tsim and Lock, 2002). Generally speaking, birds use inter-tidal areas for the following three main functions: 1) benthic fauna are food sources to birds; 2) birds "loaf", that is to say, to roost, preen, bathe and sleep on this area; 3) birds utilize mudflats for molting, which requires sufficient food resources and free from disturbance (McChesney, 1997). Mai Po Nature Reserve contains diverse habitats harboring a large variety of benthic infauna and marine invertebrates which can be consumed by migratory birds and transformed as fat and muscle tissue of the birds. The undisturbed open space of inter-tidal mudflat area in Mai Po Nature Reserve is also the ideal roosting site. Each year, hundreds of thousands of migratory birds stop at Mai Po, refuel by harvesting food and prepare for the next flights of longer distance. Located at the mid-point of East Asia-Australian Flyway, Mai Po Nature Reserve acts as an essential stop-over site and refueling ground for those migratory shorebirds (Tsim and Lock, 2002; McChesney, 1997).

Listed as a "restricted" area under the Wild Animals Protection Ordinance, Mai Po Nature Reserve is still confronted with increasing pollutions from ex situ pressure. The booming economical developments in Shenzhen Special Economic Zone accompany the potential environmental cost. Wetlands are becoming fragmented and the wildlife is disturbed. Shallow embayments are filled to reclaim more land for development. Large quantities of domestic sewage and industrial wastewater are discharged into Inner Deep Bay area posing a threat to indigenous biodiversity. The wastes from local poultry and animal farms are simply washed into the Sham Pui River and reached the Deep Bay area without any proper treatment. The data from the regular water quality monitoring program by Hong Kong SAR Government indicated that Deep Bay area is one of the most polluted water bodies in Hong Kong (Lee et al., 1999). Heavy loads of nutrients of rich organic substances exceeds what mangrove ecosystem can deal with. And the adjacent *gei wai* and fish ponds have already begun to show signs of stress and deterioration. Domestic sewage containing high concentration of organic substances is the leading cause for eutrophication, which has disturbed the original ecological balance in the Mai Po Nature Reserve. Heavy metal pollution by industrial wastes is another serious problem. Three major sources of metals available to marine invertebrate presumably are seawater, sediment and diet (Lu and Wu, 1993). It is likely that the marine invertebrates, ground-dwelling animals, fish and shrimps are under the exposure to potential accumulation of heavy metals through industrial wastewater input from Shenzhen River. Through the flow of food chain in heavy metal polluted areas of Mai Po, the toxicity will be bio-accumulated and magnified which confers potential hazards to migratory birds as advanced consumers (Lai, 2004; Lai et al., 2005). The heavy mental exposure has been reported to have negative effects on the aggressive behavior in male birds which may exert an important influence on the breeding and survival success of the birds (94). Another risk posed by environmental pollutions in Mai Po Nature Reserve is the decrease in food available to birds. As a result of water quality deterioration, there are changes in benthic species which form parts of bird's diet (Laboratory of Environmental Toxicology, 2003). Any depletion, elimination and damage of Mai Po wetlands will no doubt affect the success of bird's migration by various mechanisms such as insufficient food provision or lack of grounding place (McChesney, 1997).

In addition to the chemical and heavy metal pollution mentioned above, another aspect of domestic sewage pollutions, with perilous hazards to mankind, is the occurrence of pathogenic bacteria such as *Salmonella* and *E. coli* and infective virus (Morton and Morton, 1983). The heterotrophic bacteria brought along with domestic wastewater utilize the dissolved particulate organic matter and live freely in aquatic environment. However, through consumption of food or drinking water that has been contaminated with pathogenic bacteria or virus, human and fauna are exposed to the risk of being infected. Some may even pose life threats to public health. Other bacteria are fish pathogens which cause great economic loss in fish-farms. Therefore, the occurrence of pathogenic bacteria such as *Salmonella* and *E. coli* is a warning of the deterioration of water quality in Mai Po Nature Reserve that should receive special concerns. Moreover, besides typhoid fever caused by *Salmonella* typhi, there is another important public health problem-cholera, whose causative agent is *Vibrio cholerae* (Madigan et al., 2003a; Madigan et al., 2003b; Madigan et al., 2003c; Madigan et al., 2003d; Madigan et al., 2003e; Madigan et al., 2003f). Although there are very limited studies on the *Vibrio* species in Mai Po Nature Reserve so far, as common residents in aquatic environment of brackish water and rich suspended organic nutrition, *Vibrio* species deserve the special attention due to their potential hazards to both public and animal health.

5 Conclusions and Future Perspectives

Vibrio species can be frequently detected in a wide range of environment such as open oceans, estuaries, sediments, corals, freshwater, mollusks and fish. Though some species or strains exist as normal bacterial population in the natural marine environment, others are pathogenic or potentially pathogenic to humans, marine animals and birds. Through drinking contaminated water, consumption of contaminated seafood and direct contact with pathogen infected recreational water or wound exposure, humans are susceptible to pathogenic *Vibrio* species and confronted with health threats. The infection of marine animals by *Vibrio* species is a disaster in aquaculture, which will lead to great economic loss in mariculture farms and pose subsequent potential risks to human consumers. In addition, the coral-bleaching and large mortality of migratory birds, caused directly or indirectly by *Vibrio* species, have already warned both public and researchers worldwide about the risk from these species. Due to the fact that environmental isolates may uptake toxin-producing or antibiotic resistance genes through the plasmid-mediated conjugation process or integrase-facilitated "gene-capture", research including isolation, characterization, antibiotic resistance and plasmid profile in *Vibrio* species of environmental sources is highly desirable.

Rapid economic development and urbanization pose a serious threat to coastal ecosystem. Anthropogenic interventions through domestic sewage and industrial wastewater discharge have a great negative impact on ecosystem. The wastewater containing rich organic nutrients, heavy metals, possible bacterial pathogens and xenobiotic compounds will nourish a great number of heterotrophic bacteria including *Escherichia* and *Salmonella* and other public health threatening bacterial pathogens. Even though environmental isolates of *Vibrio* species may bear potential hazards to humans, marine-life and migratory birds, very little information is available on

the isolation and characterization of this group of emerging pathogens in the changing coastal ecosystem. Therefore, it is important that studies on the *Vibrio* species be conducted to expand our knowledge about their presence and survival in the unique ecosystem of wetland and mangrove ecosystems.

Conflict of Interest

All authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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