Chapter

Community Change and Pathogenicity of *Vibrio*

Lixing Huang, Qiancheng Gao, Youyu Zhang, Wei Xu and Qingpi Yan

Abstract

Vibrio is a rod-shaped Gram-negative bacteria, which is widely distributed in marine and estuarine environments worldwide. It is an important component of the aquatic ecosystem and plays an important role in biogeochemical cycle. Its population dynamics are usually affected by climate and seasonal factors. Most of the *Vibrios* in the environment are not pathogenic, but some of them are pathogenic bacteria for human and animal, such as *Vibrio* cholerae, *Vibrio* vulnificus, *Vibrio parahaemolyticus*, and *Vibrio* anguillarum, etc., which are generally reported to be related to aquatic animal diseases and human food-borne diseases. Over the last couple of years, due to the influence of the rising seawater temperature and climate change, the incidence of diseases caused by *Vibrio* infection has increased significantly, which poses a great threat to human health and aquaculture. The research on pathogenic *Vibrio* has attracted more and more attention. The abundance and community changes of *Vibrio* in the environment are usually controlled by many biological and abiotic factors. The *Vibrio* pathogenicity is related to the virulence factors encoded by virulence genes. The process of *Vibrio* infecting the host and causing host disease is determined by multiple virulence factors acting together, instead of being determined by a single virulence factor. In this chapter, community changes of *Vibrio*, as well as the virulence factors of *Vibrio* and the related virulence genes of Vibiro are summarized, and their important roles in *Vibrio* infection are also discussed.

Keywords: *Vibrio*, community change, foodborne diseases, pathogenicity, virulence factors

1. Introduction

The *Vibrio* belongs to the *Vibrionaceae* family of the *Gammaproteobacteria*, is a thermophilic, rod-shaped, heterotrophic Gram-negative bacterium [1]. *Vibrio* has genetic and metabolic diversity, and there are great phenotypic and genotypic differences among different species. It widely exists in estuaries and marine habitats all over the world and is an important part of aquatic ecosystems [2].

Vibrio usually has chemotaxis and motility, which can quickly respond to fluctuations of nutrient concentration and make use of nutrients in the environment to grow rapidly [3, 4]. In addition, *Vibrio* has the ability to degrade the common carbon substrates. It can decompose and utilize a variety of substrates by producing chitinase, protease, lipase and other extracellular proteases. The production and secretion of

these enzymes can provide *Vibrio* rich nutrients unavailable for other organisms, enabling *Vibrio* to quickly transform from a relatively small part to a dominant bacteria in response to environmental and climate changes [5]. *Vibrio* plays an important role in the biogeochemical cycle, which regulates inorganic nutrients and carbon flux by fixing and re-mineralize nutrients [6].

Most *Vibrio* are not pathogenic, but there are several *Vibrio* that are pathogens of humans, fish, shellfish, or other species and can cause a range of clinical manifestations including gastroenteritis, acute diarrhea, sepsis, narcotizing soft tissue infection, high mortality in cultured aquatic animals, and, in some reported cases, human death [7–10]. Vibriosis usually occurs by eating raw or under cooked seafood products, drinking contaminated water, or by direct contact with the contaminated environment through wounds [11, 12]. In the United States, the most common cause of gastroenteritis is the consumption of oysters infected or under cooked with *V. parahaemolyticus* [13]. According to the report of the Centers for Disease Control and Prevention (CDC) in 2006, *V. parahaemolyticus* is a major food-borne pathogen in the United States, and there are about 34,664 food-borne cases every year [14]. In another report, 80,000 people were infected with food-borne *Vibrio* annually in the United States in 2016, resulting in more than 500 hospitalizations and 100 deaths, the vast majority of which were *V. vulnificus* and *V. parahaemolyticus* [15]. Moreover, *V.parahaemolyticus* is a common source of food borne disease in Asian countries such as China, Japan and South Koala [7].

Aquaculture is a fast growing sector and continues to grow to meet the increasing global demand for seafood. From 2000 to 2017, aquaculture business grew by approximately 150%. China is the world's largest aquaculture producer (accounting for 58% of global production) producing 46.8 million tons of aquaculture animals per year [9]. In order to further meet the needs of the national economy and food security, the mariculture industry in southern China has gradually developed into intensive and industrialized [16]. However, high-density farming, severe human activities and global climate change have led to frequent Vibriosis, which has posed a huge threat to human health and social and economic development [17]. Vibriosis is one of the most common bacterial diseases affecting a variety of marine fish and shellfish [18, 19]. Studies have demonstrated that the content of *Vibrio* in aquaculture facilities is very high, especially during the outbreak of disease. The culturable *Vibrio* community in the affected facilities is composed of single or few *Vibrio* species, including *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *Vibrio harveyi*. These species include human and animal pathogens, which can lead to high mortality of aquatic animals [20]. For example, from May 2000 to November 2003, the mortality rate of large yellow croakers reared in marine cages due to infection with *V. alginolyticus* and *V. harveyi* ranged from 30% to 40% and even as high as 80% in Zhejiang province, China [21]. In addition, wastewater from aquaculture farms is often released to the environment without treatment, potentially causing large quantities of pathogenic *Vibrio* to enter the environment, posing a potential threat to human health. Therefore, understanding the dynamic changes of *Vibrio* community and the pathogenicity of *Vibrio* is of great significance for the healthy development of aquaculture and reducing the impact on human public health [22, 23].

2. Dynamic changes of *Vibrio* community

2.1 Abundance of *Vibrio*

Vibrio are widely distributed in estuaries and marine environments, and mainly in nearshore areas. *Vibrio* generally exhibit two different growth strategies, either

as a free-living form or attached to biological or non-biological surfaces, where they can co-exist with the host or cause host disease [24]. For example, some *Vibrio* living in squid or other organisms can be used as the source of luminescence of light-emitting organs and also an important part of the combination of biofilm and macroalgae [25].

Vibrio easily grow on conventional medium (such as seawater 2216E agar medium) and selective medium (such as thiosulfate citrate bile salt sucrose agar medium, TCBS) and can carry out a variety of metabolic activities [26]. In some studies based on culture, *Vibrio* can account for 10% of culturable marine bacteria [27], and the average abundance in estuaries and nearshore waters is 103 ~ 106 CFU L−1. However, in studies using non-culture methods, *Vibrio* population only accounts for about 1% of the total plankton bacteria in nearshore waters, and the average abundance in estuaries and nearshore waters is 10^4 ~ 10^8 16 s rRNA copies L^{-1} [28]. Their *Vibrio* abundance was found to be between 15 and 2395 CFU mL^{-1} in a study of tropical estuaries and coastal water in Malaysia [29]. In addition, studies have shown a high density of *Vibrio* on the surface and in the body of marine animals such as fish, shrimp, mollusks, corals, sponges, zooplankton, algae and seaweed [30]. For example, in a study examining the effects of aquaculture on *Vibrio* communities, the relative abundance of the 16SrRNA gene sequence reads 16 from seaweed samples were the highest by sequencing water, sediment, seaweed and tissue samples obtained in the aquaculture area of Hainan [9]. This is also consistent with studies describing *Vibrio* communities as important components of seagrass bacterial communities. These bacteria account for 25% of the culturable bacteria in seaweed off the coast of Hainan province [31].

2.2 Diversity of *Vibrio*

At least 110 *Vibrios* have been found and reported, and more may be found in the future. Among the *Vibrios* that have been described, several are commonly associated with human diseases, among which *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are recognized as human pathogenic bacteria, while *Vibrio alginolyticus*, *V. anguillarum*, *V. harveyi*, *Vibrio* fluvialis, *Vibrio* furniss, *Vibrio* metschnikovii, and *Vibrio* mimicus are primarily marine animal pathogenic bacteria but occasionally associated with human infections [32–36].

Vibrio usually has species-specific salinity and temperature preferences, and different kinds of *Vibrio* may exist in different environments. They exit from deep-sea hydrothermal vents and sediment are more than 6, 000 meters deep to seawater 10, 500 meters deep in the mariana trench [37, 38]. For example, the optimal growth temperature and salinity for *Vibrio devil*, first isolated from deep-sea hydrothermal vents, is 30 ~ 45 °C and 20 ~ 50 ppt, respectively [39]. The salinity-dependent *Vibrio* carinii is mainly present in seawater in the range of salinity from the Baltic Sea to the Mediterranean Sea [40]. *Vibrio pacinii*, *Vibrio cyclotrophicus*, *Vibrio lentus*, and some unnamed *Vibrio* have also been found at low temperatures [32].

At present, studies on the diversity of *Vibrio* communities in marine environments are mainly based on *Vibrio* isolated and cultured [41]. However, due to the low interspecific resolution of the 16S rRNA gene, the use of 16S rRNA gene similarity as a major interspecific marker for the phylogenetic relationships of *Vibrios* appears to have lost its effect. Multiple-locus sequence analysis (MLSA) and other novel phylogenetic markers such as the iron absorption regulatory gene *fur* have been used as alternative approaches [42, 43]. In order to study the diversity of environmental *Vibrio*, Siboni et al. first extracted DNA from seawater, and then used 16S rRNA gene primers specific to *Vibrio* to conduct high-throughput sequencing, thus making it possible to more intuitively and effectively explore the

diversity of *Vibrio* communities [44]. In another study by Bei et al., the abundance and community structure of *Vibrio* species at different depths was studied using *Vibrio-specific* 16S rRNA gene high-throughput sequencing and quantitative PCR (qPCR) techniques as well as traditional culture methods [5].

2.3 The influence of environmental factors on *Vibrio* community

In a marine environment, abundance and community composition of *Vibrio* is affected by many factors, including temperature, salinity, pH, water depth, dissolved oxygen and transparency [45, 46]. Chemical factors are mainly the concentrations of inorganic and organic nutrients. In addition, biological factors such as protozoa, viruses, marine animals and algae also affect the change of *Vibrio* community. Therefore, under the interaction between biological and non-biological factors, the *Vibrio* community in the environment shows complex dynamic changes.

The abundance and community structure of *Vibrio* in seawater is generally considered to be related to temperature and salinity. Temperature is the most important factor affecting the change of *Vibrio* community. Under general conditions, the relationship between *Vibrio* and water temperature shows a positive correlation. Growth of *Vibrio* population can be observed in short-term temperature rise and long-term temperature change related to climate change [47–49]. At present, many coastal areas around the world have been reported an increase in the number of *Vibrio*. For example, some researchers have used continuous plankton recording equipment to show that the increase in sea temperature has caused an increase in the number of *Vibrio* in parts of the North Atlantic and North Sea [50]. In Peru, Alaska and the gulf of Mexico and other regions also reported that due to the increase in water temperature, some pathogenic *Vibrio* species began to increase [51]. Moreover, some cases of infection caused by *Vibrio* have also been reported to be associated with abnormally high water temperatures [52].

Salinity was the second largest factor affecting the abundance of *Vibrio*, and *Vibrio* had a positive correlation with salinity, but the relationship might also be covered by increases in temperature and nutrient concentration [53, 54]. Not only that, some studies found that short-term salinity changes do increase the concentration of *Vibrio*, but long-term salinity changes have no significant effect on the overall trend of *Vibrio*, for example, there are studies found that abundance of *Vibrio* is affected by salinity and chlorophyll A concentration, but only when the salinity is less than 20 ppt, the effect of salinity is significant [29]. In another study on the abundance of microbial communities in Guanabara Bay, the researchers constructed an artificial neural network that could simulate the response of environmental microbial communities to environmental parameters. The results showed that temperature had a positive correlation with the abundance of *Vibrio*, and salinity had a negative correlation with the abundance of *Vibrio*. Transparency had a positive correlation with chlorophyll concentration but had little to do with the number of *Vibrio*. Moreover, these physical parameters were more related to the abundance of *Vibrio* than in total phosphorus and total nitrogen [55]. The authors deduced that due to the high degree of eutrophication in the bay, the microbial community had reached its maximum capacity to absorb and utilize nutrients, and the growth of the microflora was no longer restricted by nutrients. On the contrary, salinity, temperature and transparency jointly determined the number of *Vibrio*.

Although the composition and abundance of *Vibrio* communities are closely related to temperature and salinity, in temperate regions, concentrations of organic and inorganic nutrients and phytoplankton communities appear to be

more important drivers of seasonal changes in *Vibrio* communities because annual changes in temperature are not significant.

In a study of wetlands in Macchiatonda Regional Nature Reserve, it was found that the CFU abundance of TCBS depended on temperature and salinity, and the effect of temperature was greater than that of salinity (27% and 20%, respectively), but since temperature and salinity accounted for only 40% of the total CFU abundance, other environmental and biological factors had to play a role in driving *Vibrio* abundance in the system of the region [32]. In another ten-year study of the mouth of the Newz River in North Carolina, the United States, it seems that similar views have been confirmed. During the study, the temperature of the estuary did not change significantly, but the number of some *Vibrios* closely related to the temperature increased. The salinity of the estuary showed a trend of increasing to the highest and decreasing during the study. The increase of the number of *Vibrio* in the estuary had to be in conformity with the decrease in salinity. When the salinity increased, the number of *Vibrios* in the mouth of the river increased. Some specific *Vibrio*, such as *V. vulnificus*, had almost declined to undetectable levels, and the final conclusion was that the concentration of *Vibrio* in the area appeared to be independent of changes in the three factors commonly used to predict *Vibrio* abundance, including salinity, temperature, and dissolved oxygen. Although the overall abundance of *Vibrio* was on the rise, the number of some potential pathogenic species was decreasing, and the concentration of *Vibrio* in the estuaries was predicted to be related to nitrogen and carbon in the environment [2]. In addition, studies have shown that ammonium radical promotes the growth of *Vibrio*, while silicic acid and phosphate have opposite effects on *Vibrio* population [56]. Dissolved organic carbon (DOC) has a strong impact on the ecology of *Vibrio.* DOC provides a large amount of nutrients needed for *Vibrio* living in estuarine and marine habitats. *Vibrio* can absorb, metabolize and produce organic matter, thus changing its chemical properties and bioavailability [57]. Therefore, in temperate regions where the temperature is relatively stable, factors other than physical parameters such as temperature and salinity may play a more important role. However, when the degree of nutrition is high and the microbial community has reached the maximum capacity to absorb and utilize nutrition, physical factors are more relevant to the abundance of *Vibrio*.

Dissolved oxygen is an important hydrological parameter, which affects the number of *Vibrio* bacteria by affecting their metabolism. Due to hypoxia, the *Vibrio* population will switch from breathing mode to fermentation mode [58]. The abundance of free-living and particulate-related fractions of *Vibrio* was negatively correlated with dissolved oxygen (48.7% ~ 105.8% saturation) in the coastal area of georgia, USA [59]. A negative correlation between *Vibrio* abundance and dissolved oxygen (5 ~ 11 mg L^{-1}) was found in the North Carolina estuary [60]. In addition, a study on Yongle Blue Cave in Sansha, Hainan, China found that in the deepest Blue Cave in the world, due to the strong stratification limiting the vertical exchange of oxygen, the water body was divided into an upper aerobic zone and a lower anoxic zone. The strong DO gradient resulted in no significant correlation between *Vibrio* abundance and temperature, but *Vibrio* abundance was very high at a depth of 100 m (the interface between aerobic and anoxic) [5].

In addition to physical factors, biological factors also play an important role in affecting changes in the *Vibrio* community. The virus has a strong lethal effect on *Vibrio* and can greatly affect the change of *Vibrio* community. Some researchers have identified a virus with a wide host range from infected *Vibrio*, which can kill 34 *Vibrio* strains of four species [61]. For some special species, changes in biological factors may have a stronger effect on their abundance than non-biological parameters [62, 63]. Recently, it has been proved that there is a significant correlation

between the abundance of particle-associated *Vibrio* and the community composition of phytoplankton, and it is speculated that this may be related to the bioavailability of dissolved organic matter released from phytoplankton [64].

Finally, *Vibrio* can enter a viable but non-culturable state (VBNC) under adverse environmental conditions (such as oligotrophic, excessively high or low temperature, high salt, extreme pH, and sunlight radiation). This physiological state is reversible, and when the conditions become favorable again, the pathogen will recover [65]. The cells could still survive in this dormant state, but it could not be detected by the traditional culture method, which might show a higher resistance to exogenous stress and maintain the active virulence factors [66]. However, conditions for *Vibrio* to enter "recovery" from "dormancy" are not completely clear.

3. Pathogenicity of *Vibrio*

Vibrio is usually reported being related to food-borne diseases and aquaculture diseases. Diseases or even death of aquatic animals caused by *Vibrio* infection have been reported worldwide and are showing an increasing trend [7, 11, 18, 67]. Therefore, Vibriosis has caused significant impacts on human health and the development of aquaculture [68–70]. The pathogenicity of *Vibrio* is determined by multiple virulence factors encoded by its virulence genes [71–74]. *Vibrio* infects and destroys the host through a series of processes, including adhesion, invasion, immune escape, in vivo proliferation and production of toxins [46]. *Vibrio* mainly includes adhesion factor, the capsule and polysaccharide, cytotoxin and other virulence factors [75]. Therefore, infection and pathogenesis of *Vibrio* are not completed by a single virulence factor, but the result of the combined action of multiple virulence factors.

3.1 Adhesion factor

Adhesion is the prerequisite for pathogenic bacteria to cause disease to the body infection, and it is of great significance in invading the host and effectively exerting the virulence [76]. Adhesion is mainly achieved by adhesion factors that specifically recognize and bind to host cells, the ability of *Vibrio* to adhere to and form biofilm on the surfaces of organisms and non-organisms can enhance the virulence and pathogenicity of *Vibrio* [77]. Moreover, adhesion is strongly related to the biofilm formation ability, movement ability and quorum sensing of bacterial [78].

Adhesion factor is a kind of macromolecular substance that can make pathogenic bacteria adhere to the surface of eukaryotic cells, and it plays an important role in the host infection process of *Vibrio*. *Vibrio* has a variety of adhesin, such as fimbriae, cilia, outer membrane protein (OMP), lipopolysaccharide (LPS), extracellular polysaccharide, etc. Among them, fimbriae and cilia belong to fimbriae adhesin, while OMP, LPS, and extracellular polysaccharides belong to non-pilin adhesin [79]. The exposure of OMP to the bacterial surface is a unique and important component of the outer membrane of Gram-negative bacteria, which plays an important role in maintaining the outer membrane structure, ensuring the transport of substances, and stimulating the body to produce antibodies and cytokines. It has been found that the outer membrane protein is an important pathogenic factor closely related to the process of bacterial adhesion and iron uptake [80]. LPS is a lipopolysaccharide substance located in the outermost layer of the cell wall of Gram-negative bacteria. It is not only the main component of the cell wall of Gram-negative bacteria, but also the material basis for the endotoxin of the virulence factor of Gram-negative pathogens [81]. In *Vibrio* pathogens (such as *V. anguillarum*, *V.*

vulnificus, V. cholerae, *V. mimicus*, *V. parahaemolyticus*, etc.), LPS has been proven to be an important pathogenic factor [82].

Although *Vibrio* has a variety of adhesion factors, previous studies on the adhesion of *Vibrio* generally focused on flagellum, and some researchers proposed that flagellum plays an irreplaceable role in the adhesion process of bacteria-infected host [83]. According to the location of the flagellum, the flagellum can be divided into two types: terminal flagellum and peri-flagellum. Belas investigated the differences in the adsorption characteristics of *Vibrio* with different types of flagellum on chitin, and found that peri-flagellum had a stronger affinity for chitin than terminal flagellum [84]. The adhesion of *V. alginolyticus* to the epidermal mucus of *Sparus macrocephalus* was studied by Bordas, which also proved this viewpoint [85]. In addition, studies have found that pili seems to have a strong correlation with the pathogenicity of *Vibrio*. Wright first found in 1989, most clinical isolates of *V. vulnificus* have pili, while environmental isolates lack pili [86]. Analysis of pili protein gene expression during infection indicated that the pili protein expression of the strong strain was higher than that of the weak strain [87]. Moreover, the mobility of the strain was also one of the main factors affecting the adhesion. Kogure found that under the condition of having flagella at the same time, strains with mobility showed faster and stronger adhesion than the strains without mobility [88].

3.2 Capsular and polysaccharide

After entering the host, bacteria usually activate the host immune system to cause a series of immune responses to eliminate pathogens [89]. In order to survive and reproduce in the host, bacteria must adopt a series of strategies to improve their viability and virulence in the host as well as their resistance to phagocytosis and antibiotics.

The correlation between the capsular and polysaccharide and virulence has been confirmed [90]. The capsule encapsulated on the surface of bacteria is a dense, high molecular weight capsule that plays a major role in evading the host's immune defense. The encapsulated pathogen shows strong resistance to phagocytosis and complement-mediated lethality. Studies have shown that organisms with capsular polysaccharides are more likely to survive in serum, that isolates expressing opaque colonies are more resistant to serum than translucent isolates, and there are differences in colony characteristics between seafood isolates and clinical isolates. Clinical isolates were more resistant to serum complement proteins than environmental isolates, and the clinical genotype had a consistent survival advantage when exposed to serum [91–93]. In addition, the formation of biofilm will also promote the adhesion of pathogens to the host, coordinate the quorum sensing between bacteria, and improve the resistance of pathogens to antibiotics, playing a major role in the escape of pathogens from host immunity.

3.3 Cytotoxins

Cytotoxic is the main killer factor of pathogens in the process of attacking the host. Toxins secreted by *Vibrio* can be divided into endotoxin and exotoxin. Endotoxin is the lipid part of lipopolysaccharide released after cell death, and the exotoxin is secreted out of cells to cause damage to the host. At present, the toxins produced by *Vibrio* have been studied in depth, and the expression of many virulence factors is related to the pathogenicity of *Vibrio*, including: Thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH), *V. vulnificus* cytolytic toxin (VVC), cholera toxin (CT), and zonulaoccludens toxin (Zot).

V. parahaemolyticus is one of the main bacterial isolates of food poisoning caused by seafood contamination and is usually associated with outbreaks of foodborne diseases [11]. The thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) encoded by the *tdh* and *trh* genes are considered to be the main virulence factors of *V. parahaemolyticus*. TDH has a variety of biological activities, such as hemolytic activity, cytotoxicity, cardiotoxicity and enterictoxicity. TDH is a perforated toxin, and its toxic mechanism is to create pores with a diameter of 0 ~ 2 nm on the erythrocyte membrane, among which the larger pores can make the water and ions in the cells flow out of the cell membrane, and changes in these ion fluxes in the intestine are also the main cause of diarrhea [94, 95]. TRH is a thermolabile toxin, which is similar to TDH in immunology, and can also activate chloride channels and cause changes in ion flux [96]. TDH and TRH share approximately 70% homology [97], and these two genes are considered to be the most important virulence markers of *V. parahaemolyticus* and are commonly used for virulence testing of *V. parahaemolyticus* [98].

V. vulnificus is a conditionally pathogenic human pathogen, which can cause severe wound infection, acute gastroenteritis and life-threatening septicemia. In susceptible or immunocompromised individuals, the mortality rate exceeds 50% [99]. VVC encoded by *VvhA* gene of *V. vulnificus* is the key virulence factor of *V. vulnificus,* which mainly plays a role through two mechanisms: cytolysis and apoptosis induction [100]. VVC has species specificity in *V. vulnificus*, which is the only exotoxin that can be secreted out of cells, and belongs to cholesterol-dependent cytolysin of pore-forming protein family [101]. The cytotoxic mechanism of cytotoxin is that it combines with non-esterified cholesterol on cell membrane and aggregates on the cell surface, which makes the cell membrane form a channel and leads to the outflow of intracellular potassium ions, which leads to the rupture of colloid permeable cells. The main mechanism of apoptosis induced by cytotoxin is related to mitochondria. Cytotoxin can lead to the production of mitochondrial reactive oxygen species (ROS) in intestinal epithelial cells, and then lead to cell necrosis and apoptosis [102].

V. cholerae has caused several epidemics in history. Cholera toxin (CT) is the main pathogenic factor of O1/O139 *V. cholerae*, which can cause serious damage to intestinal cell function and lead to cholera watery secretory diarrhea [103]. CT is encoded by *ctxA* and *ctxB*. These genes are encoded by CTXΦ of lysogenic filamentous phage and can be transferred between virulent and non-virulent strains [104]. *zot* is also encoded by lysogenic filamentous bacteriophage CTXΦ [105], and its encoded zona-linked toxin (ZOT) is the second virulence index of *V. cholerae*, which can make mucosal cells adhere together, maintain the tight connection structure of mucosal integrity and increase the permeability of intestinal mucosa [106]. Except as a cytotoxin, Zot seems to be related to the assembly of CTX phage in structure and function. *Zot* gene has sequence homology with the coat protein gene, which is probably the coat protein of CTX Φ. This indicates that it may have dual functions [107].

3.4 Other virulence factors

In addition to secreting toxins, some pathogenic bacteria can secrete a variety of extracellular products, which are also the main factors causing host diseases. For example, Balebona and Morinigo discovered in 1995 that the extracellular products of *V. alginolyticus* have various enzyme activities such as caseinase, gelatinase, amylase, phospholipase, collagenase, etc., and these extracellular products have strong toxicity to fish cells, which can dissolve fish cells and cause fish death [108]. Balebona et al. infected fish by intramuscular injection with extracellular protease.

After 6 h, it was observed that the injected extracellular products were lysed, which could lead to fish death in 24 ~ 72 h [109]. Lee et al. found that alkaline serine protease produced by *V.* alginolyticus can reduce thrombin in prawn plasma and prevent hemolymph from agglutinating, which is one of the main lethal factors secreted by *V. alginolyticus. V. vulnificus* metalloproteinases (VVP) is a kind of zinc ion-dependent protease with hemolysis. Miyoshi et al. studies have proved that VVP can enhance vascular permeability, destroy the basement membrane, cause bleeding reaction, cause cell and tissue damage, and eventually develop into sepsis [110].

Iron, as an indispensable trace element, is also an important component of various cellular enzymes, and plays an important role in the growth, reproduction, pathogenicity and cellular metabolism of pathogenic bacteria [111]. The iron carrier of *Vibrio* is an important pathogenic factor, and the iron uptake system mediated by it plays a very important role in bacterial growth and host colonization. Under the condition of lack of iron, the strain will produce a chelation agent iron carrier with high affinity for heme iron ions and low molecular weight, and the iron absorbed from transferrin and lactoferrin will be transported to bacterial cells for its own use through the receptor [112].

4. Conclusion

Vibrio, as a kind of human and animal pathogen, exists widely in the world, and its increasing number and pathogenicity are great challenges to human public health and healthy aquaculture. The changes of *Vibrio* community in the environment are related to abiotic factors (temperature, salinity, pH, water depth, dissolved oxygen, transparency, nutrient concentration, etc.) and biological factors (protozoa, viruses, marine animals and algae, etc.), among which temperature and salinity are considered to be the most important factors affecting the changes of *Vibrio* community, but in temperate regions where salinity and temperature are relatively stable, nutrient concentration and phytoplankton community contribute more to the changes of *Vibrio* community. In recent years, with the outbreak of *Vibrio* in some areas caused by climate change and rising seawater temperature, the number of some pathogenic *Vibrio* began to increase. The pathogenicity of *Vibrio* is related to virulence factors encoded by virulence genes (adhesion factors, cytotoxins, extracellular enzymes, capsular polysaccharides, iron uptake system, etc.), and different virulence factors play different roles in the infection process. In this chapter, the influencing factors of *Vibrio* community change and various virulence factors of *Vibrio* in the process of infecting the host were summarized, in order to provide reference and help for human public health and aquaculture industry.

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