Chapter

Research Progress on Virulence Factors of *Vibrio alginolyticus*: A Key Pathogenic Bacteria of Sepsis

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Abstract

As an opportunistic pathogen, *V. alginolyticus* is commonly found in people with weak immune systems or open wounds. The history of seafood exposure is a major feature of *V. alginolyticus* infection. *V. alginolyticus* can infect marine economic animals such as fish, shrimp, and shellfish, and is also one of the key pathogens that cause sepsis in human. Because of its rapid progress and extremely high mortality after the infection, it has received more and more attention in clinical practice. At present, there is no effective method to completely control the incidence of *V. alginolyticus*. Therefore, it is particularly important to study the virulence factors and pathogenic mechanisms of *V. alginolyticus*. This article reviews recent studies on virulence factors of *V. alginolyticus*, such as quorum sensing, virulence proteins, ferroportin hemolysin, flagella, lipopolysaccharide system and biofilm formation, with the hope of providing further insights into aquaculture and public health.

Keywords: *V. alginolyticus*, sepsis, virulence, molecular mechanism

1. Introduction

Sepsis is a syndrome in which the host's response to infection is unbalanced. It refers to an acute systemic infection caused by pathogens or opportunistic pathogens that invade the blood circulation, grow and multiply in the blood, and produce toxins, which can lead to life-threatening organ dysfunction [1]. Sepsis, an epidemic disease with acute morbidity and high mortality, accounts for a large portion of global healthcare expenditures each year [2]. If sepsis is not quickly controlled, it will develop from the primary infection site to other parts of the body, causing metastatic abscesses [3], and then causing meningitis, osteomyelitis, arthritis, etc., and finally due to the accumulation of pus anywhere in the body can form an abscess, severe septic shock, and migratory lesions. Sepsis is extremely harmful to humans, and so far, no rapid and effective diagnostic biomarkers or sepsis-targeted therapeutics have been developed [4]. This may be due to the substantial heterogeneity of disease resulting from multiple underlying pathogens, sites of infection, individualized host immune responses, and manifestations of organ dysfunction [5]. Current research

shows that the pathogenic *Vibrio* that cause sepsis mainly include *Vibrio vulnificus* [6], *Vibrio cholerae* [7], and *V. alginolyticus* [8]. Among them, *V. alginolyticus*, as an emerging pathogenic bacterium, can infect aquatic animals and humans, which not only seriously affects the quality and safety of aquatic food, but also causes human disease and threatens public health.

V. alginolyticus is a globally distributed opportunistic pathogen and one of the most harmful pathogenic bacteria in marine vibriosis caused by *V. alginolyticus* has also increased, resulting in huge economic loss [9]. In recent years, with the rapid development of aquaculture, the water quality and environment of aquaculture have frequently been polluted and eutrophicated, and the number of vibriosis cases caused by *V. alginolyticus* has also increased, resulting in huge economic losses and impact on the healthy development of the aquaculture industry. *V. alginolyticus*, as one of the most abundant *Vibrio* species in marine *Vibrio*, causes *Vibrio* disease with high morbidity, high mortality and wide prevalence. Infected and then caused host disease [10]. The infection of fish by *V. alginolyticus* will cause septicemia, mainly with symptoms of bleeding, dark skin and ulcers on the skin surface [11]. Aquatic products and drinking water contaminated with *V. alginolyticus* can lead to human infection and disease outbreaks [12]. *V. alginolyticus* can not only contaminate food alone or in combination with other pathogenic bacteria to cause food poisoning, but also cause otitis media, external ear infections Inflammation, gastrointestinal diseases [13–15], etc., especially those with weak immune systems or open wounds are more susceptible to disease. According to research reports, Chien et al. believe that *V. alginolyticus* as the cause of pleural empyema and bacteremia in an immunocompromised patient [8]. Gaüzère et al. believe that *V. alginolyticus* is the pathogen of sepsis in the Indian Ocean, especially those who are engaged in marine activities are most likely to be infected by it and cause sepsis [16]. Li et al. reported the eventual death of a patient with septic shock caused by *V. alginolyticus* infection [17]. It can be seen that *V. alginolyticus* is one of the key pathogenic bacteria of sepsis.

In order to meet the diverse needs of human food, the mariculture industry is booming around the world, but the economic losses caused by vibriosis make the mariculture industry face huge challenges. *V. alginolyticus* can infect human body and fish and induce disease, causing great harm to human health and mariculture. Disease mechanism. Although *V. alginolyticus* is sensitive to most antibiotics, it is highly resistant to antibiotics such as penicillin, erythromycin, vancomycin, ampicillin, sulfisoxazole, and sulfonamides [15, 18, 19]. Shahimi [20] et al. identified the presence of four antibiotic resistance genes for penicillin (*pbp2a*), ampicillin (*blaOXA*), erythromycin (*ermB*) and vancomycin (*vanB*) by polymerase chain reaction. This is also one of the reasons for the repeated infection of *V. alginolyticus* in mariculture, which is difficult to treat, with wide damage area, long disease course and high mortality. With the increasingly strict restrictions on the use of antibiotics in modern aquaculture, there is no effective method to completely control the incidence of *V. alginolyticus*, so it is particularly important to study the virulence factors and pathogenic mechanism of the bacteria.

The pathogenic process of *V. alginolyticus* on the host body is complex and diverse. First, it adheres to the surface of host cells through adhesion. Adhesion plays a decisive role in invading host cells and causing disease. In the end, the products of its growth and metabolism contain toxins, which will directly interfere and destroy the metabolism and function of the host body [21]. This series of pathogenic processes of *V. alginolyticus* occurs under the combined action of its own environment, virulence determinants and the living conditions of the host [22, 23], in which the virulence

factor is the main manifestation of the pathogenicity of *V. alginolyticus*, and its intensity is jointly determined by the invasiveness, infectivity and pathogenicity. At present, efforts have been done to understand the pathogenic mechanism and regulatory system of *V. alginolyticus*. The main researches are about the quorum sensing system (QS) [24], virulence protein [25, 26], siderophore [27], adhesion [28], flagellar system, hemolysin and biofilm formation [29], etc. However, the specific molecular mechanism of the pathogenicity of *V. alginolyticus* virulence regulation has not been fully illustrated.

Multi-omics is the combined application of a variety of high-throughput omics technologies, mainly including gene/transcriptomics [30], proteomics [31], metabolomics [32], as well as epigenetics [33]. It is a technology that comprehensively analyzes information to clarify a certain biological mechanism. In recent years, multiomics technologies have developed rapidly in fields such as life sciences and medicine, but each individual technology cannot capture the overall view of a biological mechanism, and comprehensive multiomics analysis may be able to reveal new insights. With the rapid application of multiomics in the detection and analysis of biological samples, the reduction of time and cost required to generate these datasets, omics datasets bring both opportunities and challenges for scientists. The present review will focuse on comparative analysis of advances in genomics, transcriptomics, proteomics and metabolomics in the study of the molecular mechanism of pathogenicity of *V. alginolyticus* virulence regulation, providing new opportunities to identify new targets to help us understanding the pathogenic mechanism of *V. alginolyticus*. Here, we review recent findings based on multi-omics studies of virulence factor-related genes/proteins in *V. alginolyticus*, and provide an in-depth summary of our current understanding of the pathogenic mechanisms of *V. alginolyticus*. This review also provides guidance and suggestions for basic research and innovative perspectives on pathogenic mechanisms of *V. alginolyticus* virulence factors.

1.1 Quorum sensing system

Quorum sensing is a communication process between bacterial cells. Bacteria sense changes in population cell density by measuring the concentration of extracellular autoinducers [34]. In 1979, Nealson et al. first discovered quorum sensing in the bioluminescent marine bacterium *Vibrio fischeri* and found that quorum sensing has the function of regulating bacterial bioluminescence [35]. The bacterial population produces a certain amount of signal molecules through background expression and transports them out of the cell. With the increase of bacterial cell density, the concentration of signal molecules will also increase. When the concentration of signal molecules in the surrounding environment reaches a threshold, the QS will be triggered. The QS is an important regulatory system for the expression of *Vibrio* virulence. In the QS system of *V. alginolyticus*, LuxR and AphA homologous proteins are the two main central regulatory elements (MQSRs) that control gene expression [36]. This system mainly transmits the cell density signal in the form of phosphate groups through the AIs histidine kinase receptor into the cell, and then transmits it to the regulatory protein LuxO step by step. The phosphorylation state of LuxO determines whether Qrrs is expressed. Under the action of Hfq, Qrrs mainly regulates the expression of MQSR proteins (AphA and LuxR homologous proteins) and regulates the expression of QS pathway and QS-related biological pathway genes. AphA and LuxR homologous proteins jointly regulate 99% of the genes in the QS regulatory element [34, 37], while the rest of the genes are post-transcriptionally regulated by Qrrs

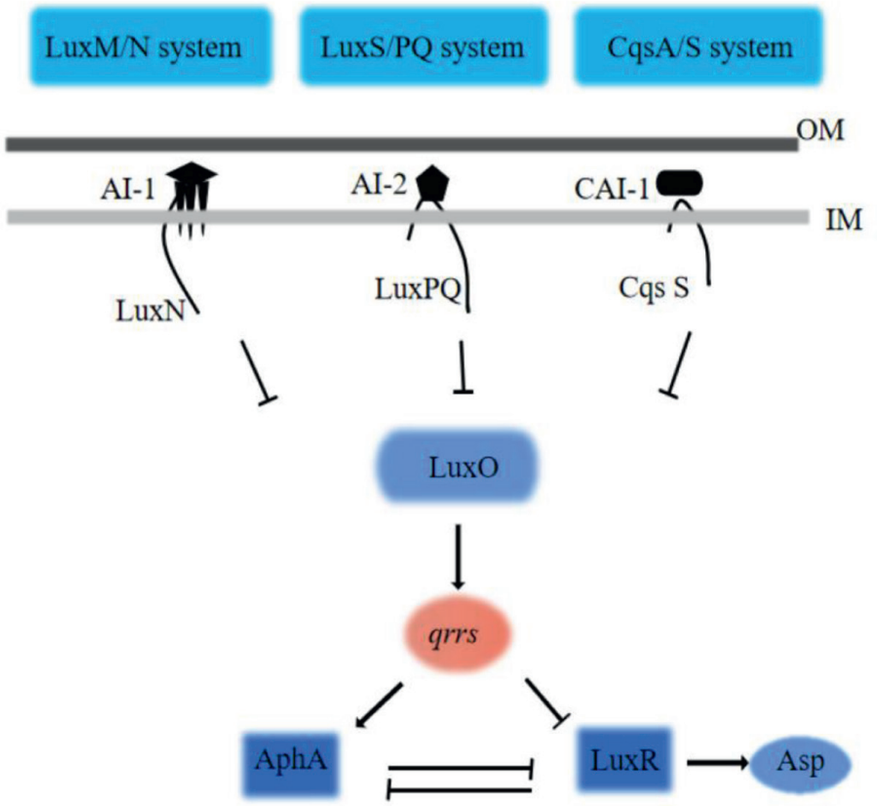

Figure 1.
Quorum sensing system of Vibrio alginolyticus.

(**Figure 1**) [38]. Therefore, in this QS system, AphA and LuxR homologous proteins are considered as central regulatory elements of quorum sensing, and their regulatory functions are conserved in *V. alginolyticus*.

In the quorum sensing system of *V. alginolyticus*, LuxR is involved in the regulation of high cell density (HCD) stage, while AphA is involved in the regulation of gene expression in low cell density (LCD) stage. AphA regulates the expression of multiple genes, especially those encoding virulence factors, flagella, motility, and biofilm formation [39, 40]. AphA negatively regulates the expression of the major virulence factor alkaline serine protease (Asp) through LuxR. Gu et al. [40] detected 49 sites rich in AphA binding peaks in the genome of *V. alginolyticus* by chromatin immunoprecipitation (ChIP) and high-throughput DNA sequencing (ChIP-seq) technology. It was demonstrated that AphA can directly regulate genes encoding adenylate cyclase (anti-σD, FabR and small RNA CsrB). At the same time, it was also revealed that AphA regulates motility through the coordinated function of LuxR and CsrB. Liu et al. [41] verified the existence of cell density-dependent sRNA Qrr in *V. alginolyticus* by transcriptomics and was homologous to QS-regulated sRNA Qrr1-5. Qrr is mainly produced under LCD and regulates the master regulators LuxR and AphA in the quorum sensing (QS) pathway at the post-transcriptional level. And both LuxR and AphA can directly bind to the promoter of qrr to activate or repress its transcription, respectively. Zhen et al. [42] found through phosphorylation proteomic studies that VstR phosphorylated by *V. alginolyticus* can enhance the expression of LuxR, thereby inhibiting T6SS1 or promoting the activity of T6SS2. In addition, the phosphatase PppA can affect the activity of QS by regulating the transcription level of LuxR, thereby regulating the expression of hcp and vgrG3 operons in T6SS.

In addition to LuxR and AphA, a third MQSR, VqsA, was found in *V. alginolyticus* through transcriptomic studies. This protein is a new LysR family transcription factor, which can regulate the biofilm formation of *V. alginolyticus*, the production of exotoxin Asp, the bactericidal ability mediated by T6SS2, and the pathogenicity to fish by directly activating LuxR and inhibiting the expression of AphA [43]. The VqsA protein shares a similar C-terminal ligand-binding domain with another LysR family protein, AphB, which is considered to be a key virulence regulator in *V. alginolyticus*. Through structural analysis, it was found that Cys189 and Cys166 may have a similar function to the thiol switch Cys235 of AphB protein, which can respond to acid stress and oxidative stress [44]. The three MQSR proteins, LuxR, AphA and VqsA, all inhibit their own expression while regulating each other, which further shows the complex regulatory network and function mediated by these factors in V. alginolyticus [43–45].

In the QS system of *V. alginolyticus*, LuxO-LuxR regulation is closely related to its virulence, by cloning another novel regulator luxT [46]. Transcription of the luxT gene is cell density-dependent, and is positively regulated by LuxU and characterized in the QS cascade to play multiple roles in regulating ECP production and motility in *V. alginolyticus*. At the same time, a new pathway, luxT, which is completely different from *Vibrio harveyi* and *Vibrio vulnificus*, was also discovered in *V. alginolyticus*. luxT can activate luxO expression at the transcriptional level as well as luxR expression at the post-transcriptional level [47]. The AhpA and LuxR proteins of *V. alginolyticus* are extremely important central regulatory elements in the quorum sensing system, and have a close regulatory relationship with virulence factors and metabolic pathways. Therefore, it is of great significance to search for regulated transcriptional regulators.

1.2 Biofilm mechanism

Most pathogenic bacteria do not exist in a single cell and planktonic state in the host's internal environment, but in the form of a group biofilm. The discovery of

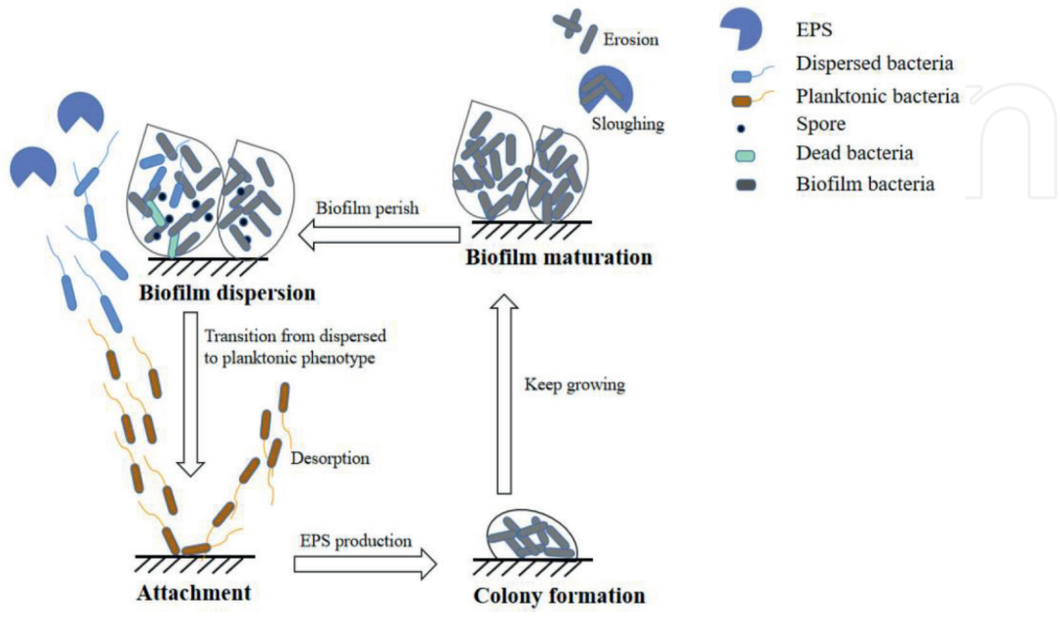

Figure 2.
Biofilm development process.

bacterial biofilms can be traced back to 1676 when Leeuwenhoek observed biofilms from his own dental plaque, but it was not a cause for concern at the time. It was not until 1978 that Costerton and others proposed the related concept of biofilm and reported the pathogenicity and drug resistance of bacterial biofilm, and the cell membrane attracted the attention of scholars [48, 49]. BF is a bacteria adhesion to the surface of inert or active objects, and surrounded by polysaccharide matrix, protein, lipids and other extracellular matrices (EPS), forming a highly organized, systematic membrane-like polymer with a special complex structure [50]. BF formation is a dynamic process closely related to exopolysaccharide production, cell migration, subpopulation differentiation and interactions (**Figure 2**). Studies show that the formation process of BF of *V. alginolyticus* mainly includes four processes: (i) Bacteria attach to biotic or abiotic surfaces; (ii) Bacteria lose their ability to move and begin to produce biofilm substrates, forming biofilms; (iii) Biofilm maturation and thickening; (iv) Biofilm diffusion (with the aging of the biofilm, bacteria disperse from the biofilm and revert to a planktonic state, and the life cycle begins again) [51, 52].

Bacterial biofilms are an important mechanism leading to bacterial resistance and are associated with most bacterial infections. According to CDC statistics, 65% of human bacterial infections are related to the formation of biofilms [53]. Biofilms make bacteria in biofilms significantly more resistant to antibiotics and disinfectants than planktonic bacteria through a series of different mechanisms. For example, the impermeability of the biofilm matrix to antibiotics, the reduced growth rate of bacteria at the core of the biofilm, the presence of antibiotic-resistant persister bacteria, and the overexpression of bacterial efflux pumps in the biofilm, causing persistent infection, are the main reasons for treatment failure [54]. RpoN has been reported to be involved in *Vibrio* biofilm formation, and RpoN (σ54) acts as a global regulator to control key virus-associated phenotypes, which can regulate flagella and extracellular polysaccharides (EPS) during pathogenic biofilm formation. Zhang et al. showed that when the *rpoN* gene in *Vibrio alginolyticus* is deleted, it will lead to the failure of biofilm detachment, the loss of flagella and the reduction of motility, which proves that *rpoN* is crucial for the separation of *V. alginolyticus* biofilms [55]. Gu et al. concluded through RNA-seq and ChIP-seq that RpoE and RpoX have different regulators, regulating a total of 105 genes under high temperature conditions, including those related to biofilm formation, motility, virulence and other related genes, meanwhile, it was proved that RpoX can up-regulate genes related to flagella, biofilm formation and hemolytic activity at higher temperature [56].

Some studies have found [57, 58] that bacterial biofilm formation is closely related to its swarm effect, and swarm effect mutants cannot form normal mature biofilm structures, and their resistance to drugs and host immune systems is significantly reduced. Stringent responses mediated by the bacterial alarmins pppGpp and ppGpp [collectively (p)ppGpp] are considered important adaptive responses to stressful conditions. Yin Wenliang et al. showed that in *V. alginolyticus*, the (p)ppGpp synthase genes *relA* and *spoT* can contribute to biofilm formation at low cell density and biofilm formation at high cell density, respectively. It was also shown that (p)ppGpp could serve as a key regulator of *V. alginolyticus* biofilm formation [59]. As a three-phase ATP-independent periplasmic transporter solute-binding subunit, DctP. Zhang et al. constructed a *dctp* mutant strain, compared with the wild strain of *V. alginolyticus*, its biofilm formation ability was significantly reduced. Further transcriptomic analysis showed that the deletion mutation of *dctp* could regulate the expression levels of 22 related genes in the colonization, adhesion and pathogenicity of *V. alginolyticus* [60]. Oligopeptide permease (Opp) has now been shown to play an important role in

the virulence of *V. alginolyticus*. Liu et al. identified the *opp* gene cluster in the transcriptome sequence of *V. alginolyticus*, and proved that *oppABCDF* has an important contribution to the biofilm formation of *V. alginolyticus*, among which *oppA* and *oppB* have the greatest influence, and *oppF* has the minimal impact [29]. The *toxR* is a conserved virulence-related gene in *Vibrio*. Chen et al. found that the deletion of *toxR* can significantly reduce the biofilm formation ability of *V. alginolyticus*. The analysis of outer membrane protein (OMP) showed that ToxR may enhance the formation of biofilm by regulating the production of OMP [61]. In addition, EPS can immobilize microbial communities in biofilms and maintain a series of highly complex dynamic changes that play important roles in both biofilm structure and function, including surface adhesion, spatial and chemical heterogeneity, biofilm virulence composition, attenuates the effects of antimicrobial agents, and promotes cell-to-cell interactions, thereby enhancing the metabolic capacity and drug resistance of cells in biofilms [62].

Due to different microbial species, local shear stress, nutrient and substrate utilization, and host environment, biofilm EPS matrix composition and structure are quite different [50]; moreover, the secretion and spatial organization of EPS also differ between single and multiple bacterial communities [62]. Microbial species and metabolic activity, nutrient availability, host environment, and growth stage all affect the variability of EPS composition and structure, making it difficult to develop EPS-targeting drugs and technologies. Various EPS substrates secreted by *V. alginolyticus* play important roles in maintaining the stability and scaffolding of biofilm structures, and are also involved in biofilm assembly, population behavior, and functional properties of toxicity, such as signaling, genetic exchange, microenvironment formation, mechanical stability, antibiotic resistance, etc. Although some studies have revealed the composition and function of EPS matrix in the biofilm system of some model microorganisms, the underlying mechanisms of complex signaling molecules and biofilm formation and virulence factor expression still need to be elucidated.

1.3 Siderophore system

Iron is an essential element for the pathogenic process of *V. alginolyticus* and plays an important role in the life activity of *V. alginolyticus*. Although iron is abundant in nature, in the host body it is usually bound to proteins to form complexes, and the level of iron ions free outside the cells is very low [63, 64]. In order to obtain iron in the host, pathogenic pathogens such as *V. alginolyticus* have developed two iron uptake systems, one that destroys erythrocytes in host tissues through exotoxins and then obtains iron ions from heme released from erythrocytes, and the other that produces high-affinity iron carriers (siderophores) that can transport iron taken up from transferrin and lactoferrin to bacteriophage cells for their own use through transport [63].

The ability to take up available dissolved iron ions from the environment is essential for the growth of most bacteria in an iron-limited environment. In bacteria, iron uptake mechanisms undertaken by iron carriers have evolved for this purpose. Genes for the synthesis and transport of iron carriers are usually present in chromosomes or plasmids. Two groups of enzymes, non-ribosomal peptide synthase (NRPS) and NRPS-independent synthase (NIS) [65], are responsible for iron carrier biosynthesis [66]. In Gram-negative bacteria, the iron-iron carrier complex is recognized by speciWc tonb-activated outer membrane receptors and then transferred to successive components, such as periplasmic binding proteins and permease proteins [67]. In most cases, the expression of genes required for the synthesis and transport of iron carriers is readily regulated by a combination of biologically effective iron

concentrations and iron uptake regulatory proteins (Fur) to maintain optimal intracellular iron growth at a relatively stable concentration [68]. Among other pathogenic *Vibrio* species, the strong iron carrier-mediated iron acquisition system is considered to be one of the important virulence advantages [69].

Wang et al. [27] isolated from the pathogenic bacterium Vibrio MVP01, a cluster of 11 genes consisting of two differentially transcribed manipulators pvsABCDE and psuA-pvuABCDE, regulated by Fe^{3+} and iron uptake regulators (Fur), with high similarity to loci associated with iron carrier biosynthesis and transport in *Vibrio parahaemolyticus*. When single in-frame mutations occur in *pvsA* and *pvsD* of the *pvsABCDE* manipulator or *pvuA*, *pvuB* and *pvuE* of the *psuA-pvuABCDE* manipulator, they prevent the biosynthesis or utilization of algal iron carriers, indicating that they play an important role in the biosynthesis or utilization of iron carriers in *V. alginolyticus* MVP01. Addition of purified iron carriers restored cell growth of iron carrier biosynthesis mutants, but not iron carrier uptake mutants [27]. Wang et al. [24] cloned the *luxO* gene from *V. alginolyticus* MVP01 through genomic studies, and gene analysis showed that it encodes a protein highly similar to other LuxO homologs. *luxO* in-frame deletion mutants and *rpoN* null mutants were constructed with suicide plasmids, demonstrating that deletion of LuxO alone increased extracellular protease and hemolysis product secretion, but reduced *V. alginolyticus* MVP01 production of iron carriers. The *rpoN* deletion mutants showed significantly higher protease levels and iron carrier production, and significantly lower hemolytic activity of ECP. *V. alginolyticus* has a functional *luxO* gene that regulates the secretion of extracellular proteases and hemolytic substances and the production of iron carriers in an r54-dependent or independent manner. *V. alginolyticus* MVP01 produces extracellular proteases and hemolytic active substances as well as iron carriers, which may characterize the virulence of this strain. Uncovering the regulation of the secretion of these products by LuxO and r54 and the potential population sensing system of *V. alginolyticus* MVP01 will contribute to the understanding of the pathogenesis of *Vibrio* [24].

1.4 Flagellar system

Bacterial motility is a mechanism shared by many microorganisms and is necessary for pathogenic bacteria to invade their hosts and achieve their life activities; therefore, it is treated as an important virulence factor for many pathogens [70], and the flagellum is one of the main motility organs of bacteria, while the number and location of flagella vary depending on the bacterial species [71]. *V. alginolyticus* contains two unique flagellar systems, (i) a polar flagellum with a membrane sheath suitable for swimming in a liquid environment and (ii) a large number of lateral flagella suitable for aggregation on the surface of living and nonliving objects and produced only when the polar flagellum is not functional [72, 73].

The LuxS population sensing system has been reported to regulate the expression of several virulence factors of pathogenic bacteria. Inactive *luxS* results in reduced virulence of *V. alginolyticus*. A *luxS*-deficient mutant presents a defect in motility and flagellogenesis, which is compensated by intact *luxS.* Since motility depends on flagella, genes related to *V. alginolyticus* flagellogenesis, including flagellar regulatory genes *flaK* and *lafK* and secondary flagellar genes *fliS* and *lafA*, were cloned and characterized by real-time fluorescence quantitative reverse transcription polymerase. The differential expression of these genes in wild-type and *luxS* mutants was confirmed by real-time fluorescence quantitative reverse transcription polymerase chain reaction. The results indicate that LuxS plays an important regulatory role in

motility and flagellar biogenesis in *V. alginolyticus* [74], and the study of the role of *luxS* in kinetic regulation is important for understanding the virulence mechanism of *V. alginolyticus*.

V. alginolyticus usually has unipolar flagella whose number and position are positively regulated by FlhF. Shota Kondo et al. [75] introduced random mutations into FlhF through genomics in mutants that fail to produce flagella at the cell pole, and these new mutations were localized only to the GTPase motif of FlhF. Compared to wild type, mutant FlhF proteins have reduced polar localization and can still bind to membranes. The results suggest that the GTPase motif of FlhF plays a key role in the polar localization of the protein during flagellar formation.

FlhF is a GTPase that is a homolog of the signal recognition particle (SRP) protein Ffh and the SRP receptor FtsY. FlhF is located at the cell poles and directs flagellum formation. FlhF and FlhG are proteins that control flagellum formation in bacteria with flagella at both cell poles [76–78]. *flhF* is involved in determining the location of flagellum formation [79, 80]. *V. alginolyticus* has only one flagellum at the cell pole, and FlhF overexpression or FlhG deficiency leads to the formation of multiple flagella at the cell pole. When FlhF is deficient or FlhG is overexpressed in the cell, the cell has no flagellum. Thus, FlhF and FlhG negatively and positively control the number of flagella, respectively. Furthermore, co-expression of FlhF and FlhG reduces the number of polar flagella more than FlhG alone, suggesting that FlhG co-regulates the number of polar flagella with FlhF. To investigate the interaction between FlhF and FlhG, Akiko Kusumoto et al. [80], using anti-FlhF antibodies for immunoprecipitation, proposed a model in which the localization of FlhF at the pole determines the polar position and flagellar production, and FlhG interacts with FlhF to prevent FlhF from polar positioning, and thus FlhG negatively regulates the number of flagella in *V. alginolyticus* cells.

Zhu [81] et al. studied the sheath flagellum of *V. alginolyticus* by a combination of cryoelectron tomography (cryo ET) and subspectral analysis as well as genetic methods, revealing significant differences between *V. alginolyticus* cells with and without sheath flagella, which have a ring-like structure at the base of the hook that is associated with major remodeling of the outer membrane and sheath formation.

1.5 Hemolysin

Hemolysin, an exotoxin that lyses the erythrocyte membrane by releasing hemoglobin, is the most widely distributed toxin in pathogenic *Vibrio* [82]. Hemolysin acts on the erythrocyte membrane, leading to cell lysis, which in turn releases iron-binding proteins, such as hemoglobin, transferrin and lactoferrin. Iron can then be obtained through a high-affinity iron acquisition system capable of competing with host iron-binding proteins. The main system centers on iron carriers, which are low molecular weight chelators that specifically bind extracellular Fe^{3+} and are subsequently taken up through receptors on the cell membrane [83–85]. Hemolysin expression in Vibrio maritimus is regulated under iron-limited conditions and occurs in the host during infection [84]. In many cases, the hemolysin pore-forming activity is not limited to erythrocytes, but extends to a wide range of other cell types, including mast cells, neutrophils and polymorphonuclear cells, and enhances toxicity by causing tissue damage [86]. There are five representative families of hemolysins in Vibrio, including the heat-resistant direct hemolysin (TDH) family, the HlyA (or E1 Tor hemolysin) family, the heat-resistant hemolysin (TLH) family, the heat-resistant hemolysin (δ-VPH) family, and the novel hemolysin gene (HLX) family [87].

TDH and HlyA have been extensively studied and are closely associated with virulence [87, 88]. However, the role of some other hemolysins, such as TLH, δ-VPH and HLX, is unknown and needs to be determined by further studies.

1.6 Virulence proteins

V. alginolyticus can resist environmental and host-killing effects in many ways. Biofilm formation is one of the important ways. Biofilm formation can enhance host damage by secreting extracellular virulent proteases and exotoxins [89]. At present, the research on the pathogenic mechanism of *V. alginolyticus* has been widely carried out, which is similar to the pathogenic factors of *V. parahaemolyticus* [90]. Its pathogenicity involves many virulence factors, such as heat-resistant direct hemolysin protein (TDH), heat-resistant related hemolysin protein (TRH), type three secretion system (T3SS) and type three secretion system effectors (T3SES) [91, 92].

Studies have shown that the virulence of *V. alginolyticus* is related to the extracellular protease genes *proA* and *vacB*, hemolysin genes *tlh*, *tdh* and *trh*, iron uptake system-related genes tonB-exbB-exbD and density sensing genes *luxO*, *luxS* and *luxR* [93]. Cheng Haiyan studied the virulence-related genes of *toxR* and *acfA*, but the effects of *toxR* and *acfA* genes on the expression of major virulence proteins were not significant [94]. Quan Taishu reported that the same hemolysin TDH as *V. parahaemolyticus* was detected from *V. alginolyticus*, and 86. 0% of the existing strains were positive in Kanagawa test, which proved that the pathogenicity of *V. alginolyticus* was similar to that of *V. parahaemolyticus* [95].

Most of these virulence factors exist in the form of protein, which injects toxin proteins into the host body through the transmembrane transport organs of *vibrio*, such as the secretion system and the two-component system, thus leading to the disease of the body [96]. The type III secretion system is a tightly controlled virulence mechanism that is used for colonization by many Gram-negative bacteria. T3SS consists of cytoplasmic bulbs, a matrix spanning the inner and outer membranes of the cell membrane, and extracellular needles. When infecting the host, T3SS can secrete the virulent protein to the outside of the cell or the surface of the host cell, and even directly "inject" into the host cell and finally cause the death of the host cell [97]. Effector proteins or virulence proteins, the embodiment of bacterial virulence, are directly secreted out of cells or transported to host cells. At present, studies on effector proteins of *V. alginolyticus* type III secretion system are rare. Pang et al. constructed Δ*hopPmaJ*. Compared with wild strains, the virulence, adhesion and swimming ability of Δ*hopPmaJ* were significantly decreased [98]. Zhou et al. constructed Δ*typeA*, which exhibited a significantly reduced toxicity to zebrafish compared with wild strains. Analysis showed that Δ*typeA* could enhance the expression of immune genes such as IgM and IL-1β in fish. It could be demonstrated that by regulating the expression of T3SS gene, it affected the secretion and transport of its effector protein, thereby affecting the pathogenicity of vibrio [98, 99].

1.7 LPS system

LPS is the main component of cell wall, which consists of three parts: core polysaccharide, lipid A and O-specific side chain, and mainly exists in gram-negative bacteria [100, 101]. Among them, lipid A is the toxic part of LPS, which can cause non-specific physiological and pathological reactions in the host, such as fever,

disseminated intravascular coagulation, hypotension, leukocyte reaction, and shock. At the same time, LPS is also involved in the process of bacterial adhesion, invasion, and host cell diffusion [102].

Jian et al. immunized grouper with *V. alginolyticus* LPS, and found that LPS can stimulate fish to produce good humoral immune response, and can also significantly improve the activity of lysozyme, antibacterial activity and SOD in experimental fish serum [103]. Therefore, *V. alginolyticus* LPS can be used as a good immunogen, and can also be used as an immunopotentiator to improve the anti-infection ability of fish, which can play an important role in preventing and treating fish diseases. Yan et al. used the lipopolysaccharide solution freeze-dried powder separated from *V. alginolyticus* solution to inject the large yellow croaker with the injection concentration of 0.5 mg/ml. The large yellow croaker died within 5 days. When the concentration was increased to 2 mg/ml, the mortality of the large yellow croaker reached 100% within 3 days [104]. Xu et al. injected lipopolysaccharide of *V. alginolyticus* with different concentrations into *Epinephelus coioides*. The experimental results showed that the immunogenicity of LPS of *V. alginolyticus* on *E. coioides* was enhanced with the increase of LPS concentration, and the immunogenicity of *E. coioides* was also enhanced [105, 106].

It has been found that LPS plays a very important role in the pathogenesis of infection [107]. Zhou et al. studied the structure of Lipid A of *V. parahaemolyticus* and related genes. By gene comparison, they found that there were four genes in *V. parahaemolyticus* ATCC33846 that might be related to the secondary acylation during the synthesis of LIPID A: VP_RS01045, VP_RS00880, VP_RS08405 and VP_RS12170, which provides a further research direction for further research on the structure of the lipopolysaccharide Lipid A of *V. alginolyticus* [108].

2. Conclusions

To sum up, the infection of *V. alginolyticus* is the result of the joint action of multiple virulence factors, which is in line with the general mechanism of pathogenic bacteria. *V. alginolyticus* can cause damage to the tissues of the organism during the invasion and proliferation of the host, and the metabolites produced in this process can further damage the organism or disturb the normal metabolism of the host organism, thereby causing serious outbreaks of diseases in aquatic animals and humans. *V. alginolyticus* infection can range from mild gastroenteritis to necrotizing soft tissue infection, followed by sepsis. For a long time, the prevention and treatment of *Vibrio* disease has been a worldwide problem. Although the use of antibiotics to prevent and control *V. alginolyticus* has certain results, the long-term abuse of antibiotics or drugs will pollute the water environment and lead to the emergence of drug-resistant strains, making the disease control more difficult. Therefore, studying the pathogenic mechanism of septicemia caused by *V. alginolyticus* can provide a relatively comprehensive understanding of *V. alginolyticus*.

Although some known virulence factors of *V. alginolyticus* have been identified, the mechanism of sepsis caused by *V. alginolyticus* remains unclear, and individuals infected with *V. alginolyticus* are still at high risk of sepsis. Combined with the current development trend, the use of gene sequencing and quantitative proteomics, high-throughput screening technology and bioinformatics analysis to identify the potential pathogenic genes and virulence factors of *V. alginolyticus* will be the future research direction.

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References

[1] Rudd KE, Kissoon N, Limmathurotsakul D, Bory S, Mutahunga B, Seymour CW, et al. The global burden of sepsis: Barriers and potential solutions. Critical Care. 2018;**22**(1):1-11

[2] Reyes M, Filbin MR, Bhattacharyya RP, Billman K, Eisenhaure T, Hung DT, et al. An immune-cell signature of bacterial sepsis. Nature Medicine. 2020;**26**(3):333-340

[3] Reducing the global burden of sepsis: A positive legacy for the COVID-19 pandemic? Intensive Care Medicine. 2021:733-736

[4] Filbin MR, Lynch J, Gillingham TD, Thorsen JE, Pasakarnis CL, Nepal S, et al. Presenting symptoms independently predict mortality in septic shock: Importance of a previously unmeasured confounder. Critical Care Medicine. 2018;**46**(10):1592-1599

[5] Seymour CW, Kennedy JN, Wang S, Chang CCH, Elliott CF, Xu Z, et al. Derivation, validation, and potential treatment implications of novel clinical phenotypes for sepsis. JAMA. 2019;**321**(20):2003-2017

[6] Sheer AJ, Kline KP, Lo MC. From sea to bloodstream: Vibrio vulnificus sepsis. The American Journal of Medicine. 2017;**130**(10):1167-1169

[7] Shashindran N, Narendrakumar L, Udayakumaran S, Vijayakumar DM, Thomas S, Kumar A. First report of Vibrio cholerae O9, novel st520, isolated from a child with bacteraemia-associated sepsis. Indian Journal of Medical Microbiology. 2020;**38**(3-4):489-491

[8] Chien J, Shih J, Hsueh P, Yang P, Luh K. Vibrio alginolyticus as the cause of pleural empyema and bacteremia in an immunocompromised patient. European Journal of Clinical Microbiology and Infectious Diseases. 2002;**21**(5):401

[9] Colwell RR, Grimes DJ. Vibrio diseases of marine fish populations. Helgoländer Meeresuntersuchungen. 1984;**37**(1-4):265-287

[10] Cai SH, Huang YC, Lu YS, Wu ZH, Wang B, Tang JF, et al. Expression and immunogenicity analysis of accessory colonization factor A from Vibrio alginolyticus strain HY9901. Fish & Shellfish Immunology. 2013;**34**(2):454-462

[11] Lee KK. Pathogenesis studies on Vibrio alginolyticus in the grouper, Epinephelus malabaricus, Bloch et Schneider. Microb Pathog. 19 Jul 1995;**19**(1):39-48

[12] Wentworth, Berttina B. "Diagnostic procedures for bacterial infections." Diagnostic procedures for bacterial infections. 1987;868-868

[13] Prieto JR, Palazón JH. Otitis media due to Vibrio alginolyticus: The risks of the Mediterranean Sea. Anales Espanoles de Pediatria. 1993;**39**(4):361-363

[14] Ardiç N, Ozyurt M. Case report: Otitis due to Vibrio alginolyticus. Mikrobiyoloji Bulteni. 2004;**38**(1-2):145-148

[15] Mukherji A, Schroeder S, Deyling C, Procop GW. An unusual source of vibrio alginolyticus–associated otitis: Prolonged colonization or freshwater exposure? Archives of Otolaryngology–Head & Neck Surgery. 2000;**126**(6):790-791

[16] Gaüzère BA, Chanareille P, Vandroux D. Post nearly drowning Vibrio

alginolyticus septicemia acquired in reunion (Indian Ocean). Bulletin de la Societe de Pathologie Exotique (1990). 2016;**109**(3):151-154

[17] Lee DY, Moon SY, Lee SO, Yang HY, Lee HJ, Lee MS. Septic shock due to Vibrio alginolyticus in a cirrhotic patient: the first case in Korea. Yonsei Medical Journal. 2008;**49**(2):329-332

[18] Menezes FGD, Rodriguez MT, Carvalho FCD, Rebouças RH, Costa RA. Pathogenic Vibrio species isolated from estuarine environments (Ceará, Brazil)-antimicrobial resistance and virulence potential profiles. Anais da Academia Brasileira de Ciências. 2017;**89**:1175-1188

[19] Damir K, Irena VS, Damir V, Emin T. Occurrence, characterization and antimicrobial susceptibility of Vibrio alginolyticus in the Eastern Adriatic Sea. Marine Pollution Bulletin. 2013;**75**(1-2):46-52

[20] Shahimi S, Elias A, Salami M, Fauzi F, Ghani A, Azuhairi A. Antibiotic resistance and determination of resistant genes among cockle (Anadara granosa) isolates of Vibrio alginolyticus. Environmental Science and Pollution Research. 2021;**28**(32):44002-44013

[21] Huang L, Hu J, Su Y, et al. Identification and characterization of three V. *alginolyticus* non-coding RNAs involved in adhesion, chemotaxis, and motility processes. Frontiers in Cellular and Infection Microbiology. 2015;**5**:56

[22] Lee KK, Yu SR, Yang TI, et al. Isolation and characterization of V. alginolyticus isolated from diseased kuruma prawn, Penaeus japonicus. Letters in Applied Microbiology. 1996;**22**(2):111-114

[23] Bejarano PA, Langeveld JP, Hudson BG, et al. Degradation of basement membranes by Pseudomonas aeruginosa elastase. Infection and Immunity. 1989;**57**(12):3783-3787

[24] Wang Q, Liu Q, Ma Y, Rui H, Zhang Y. LuxO controls extracellular protease, haemolytic activities and siderophore production in fish pathogen Vibrio alginolyticus. Journal of Applied Microbiology. 2007;**103**(5):1525-1534

[25] Balebona MC, Andreu MJ, Bordas MA, Zorrilla I, Moriñigo MA, Borrego JJ. Pathogenicity of Vibrio alginolyticus for cultured gilt-head sea bream (Sparus aurata L.). Applied and environmental microbiology. 1998;**64**(11):4269-4275

[26] He H, Wang Q, Sheng L, Liu Q, Zhang Y. Functional characterization of Vibrio alginolyticus twin-arginine translocation system: its roles in biofilm formation, extracellular protease activity, and virulence towards fish. Current Microbiology. 2011;**62**(4):1193-1199

[27] Wang Q, Liu Q, Ma Y, Zhou L, Zhang Y. Isolation, sequencing and characterization of cluster genes involved in the biosynthesis and utilization of the siderophore of marine fish pathogen Vibrio alginolyticus. Archives of Microbiology. 2007;**188**(4):433-439

[28] Luo G, Huang L, Su Y, et al. flrA, flrB and flrC regulate adhesion by controlling the expression of critical virulence genes in V. alginolyticus. Emerging Microbes & Infections. 2016;**5**(8)e00511

[29] Liu W, Huang L, Su Y, et al. Contributions of the oligopeptide permeases in multistep of V. alginolyticus pathogenesis. MicrobiologyOpen. 2017;**6**(5):e00511

[30] Beer BK. Bethesda, and biology: How "genomics" came into being. Journal of the National Cancer Institute. 1998;**90**(2):93

[31] Celis JE, Rasmussen HH, Gromov P, et al. The human keratinocyte two-dimensional gel protein database (update 1995): Mapping components of signal transduction pathways. Electrophoresis. 1995;**16**(1):2177-2240

[32] Nicholson JK, Lindon JC. Systems biology: Metabonomics. Nature. 2008;**455**(7216):1054-1056

[33] Hong YU. Epigenetics: Advances of non-coding RNAs regulation in mammalian cells: Epigenetics: advances of non-coding RNAs regulation in mammalian cells. Hereditas (Beijing). 2009;**31**(11):1077-1086

[34] Rutherford ST, Van Kessel JC, Shao Y, et al. AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. Genes & Development. 2011;**25**(4):397-408

[35] Nealson KH, Hastings JW. Bacterial bioluminescence: Its control and ecological significance. Microbiological reviews. 1979;**43**(4):496-518

[36] Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nature Reviews. Microbiology. 2016;**14**(9):576-588

[37] Van Kessel JC, Rutherford ST, Shao Y, Utria AF, Bassler BL. Individual and combined roles of the master regulators AphA and LuxR in control of the Vibrio harveyi quorum-sensing regulon. Journal of Bacteriology. 2013;**195**(3):436-443

[38] Shao Y, Feng L, Rutherford ST, Papenfort K, Bassler BL. Functional determinants of the quorum-sensing non-coding RNAs and their roles in target regulation. The EMBO Journal. 2013;**32**(15):2158-2171

[39] Lu R, Osei-Adjei G, Huang X, et al. Role and regulation of the orphan AphA protein of quorum sensing in pathogenic Vibrios. Future Microbiology. 2018;**13**:383-391

[40] Gu D, Liu H, Yang Z, Zhang Y, Wang Q, et al. Chromatin immunoprecipitation sequencing technology reveals global regulatory roles of low-cell-density quorum-sensing regulator AphA in the pathogen V. alginolyticus. Journal of Bacteriology. 2016;**198**(21):2985-2999

[41] Liu H, Liu W, He X, et al. Characterization of a cell density-dependent sRNA, Qrr, and its roles in the regulation of the quorum sensing and metabolism in V. alginolyticus. Applied Microbiology and Biotechnology. 2020;**104**(4):1707-1720

[42] Yang Z, Zhou X, Ma Y, et al. Serine/threonine kinase PpkA coordinates the interplay between T6SS2 activation and quorum sensing in the marine pathogen V. alginolyticus. Environmental Microbiology. 2018;**20**:903-919

[43] Xiating G, Xuetong W, Qiaoqiao M, et al. VqsA, a Novel LysR-Type transcriptional regulator, coordinates quorum sensing (QS) and is controlled by QS to regulate virulence in the pathogen V. alginolyticus. Applied and Environmental Microbiology. 2018;**84**(12):e00444-18

[44] Gao X, Liu Y, Liu H, et al. Identification of the regulon of AphB and its essential roles in LuxR and exotoxin Asp expression in the pathogen V. alginolyticus. Journal of Bacteriology. 2017;**199**(20):e00444-18

[45] Gu D, Guo M, Yang M, et al. A σE-mediated temperature gauge controls a switch from LuxR-mediated virulence gene expression to thermal stress adaptation in V. alginolyticus. PLoS Pathogens. 2016;**12**(6):e1005645

[46] Rui H, Liu Q, Ma Y, et al. Roles of LuxR in regulating extracellular alkaline serine protease A, extracellular polysaccharide and mobility of V. alginolyticus. FEMS Microbiology Letters. 2008;**285**(2):155-162

[47] Liu H, Gu D, Cao X, et al. Characterization of a new quorum sensing regulator luxT and its roles in the extracellular protease production, motility, and virulence in fish pathogen V. alginolyticus. Archives of Microbiology. 2012;**194**(6):439-452

[48] Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Scientific American. 1978;**238**(1):86-95

[49] Costerton JW, Geesey GG, Cheng KJ, et al. Bacterial biofilms in nature and disease. 1987;**41**:435-464

[50] Flemming HC, Wingender J, Szewzyk U, et al. Biofilms: An emergent form of bacterial life. Nature Reviews. Microbiology. 2016;**14**(9):563-575

[51] Muhsin J, Wisal A, Saadia A, et al. Bacterial biofilm and associated infections. Journal of the Chinese Medical Association. 2018;**81**(1):7-11

[52] Gang Y, Yingxue Q, Wenzheng Z, et al. Study on biofilm formation characteristics of pathogenic V. alginolyticus. Aquatic Science. 2012;**31**(02):73-78

[53] Cvitkovitch DG, Li Y, Ellen RP. Quorum sensing and biofilm formation in Streptococcal infections. The Journal of Clinical Investigation. 2003;**112**(11):1626-1632

[54] Romling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of Internal Medicine. 2012;**272**(6):541-561

[55] Na Zhang, Shun Zhang, Wei Ren, et al. Roles of rpoN in biofilm formation of V. alginolyticus HN08155 at different cell densities. Microbiological Research, 202;**247**:126728

[56] Gu D, Zhang J, Hao Y, et al. Alternative sigma factor RpoX is a part of the RpoE regulon and plays distinct roles in stress responses, motility, biofilm formation, and hemolytic activities in the marine pathogen V. alginolyticus. Applied and Environmental Microbiology. 2019;**85**(14):e00234-19

[57] Petersen FC, Pecharki D, Scheie AA. Biofilm mode of growth of Streptococcus intermedius favored by a competence-stimulating signaling peptide. 2004;**186**:6327-6331

[58] Davies DG, Parsek MR, Pearson JP, et al. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science (New York, N.Y.). 1998;**280**(5361):295-298

[59] Wen Liang Y, Zhen Yu X, Yan Hua Z, et al. Two (p)ppGpp synthetase genes, relA and spoT, are involved in regulating cell motility, exopolysaccharides production, and biofilm formation of V. alginolyticus. Frontiers in Microbiology. 2022;**13**:858559

[60] Zhang Y, Tan H, Yang S, et al. The role of dctP gene in regulating colonization, adhesion and pathogenicity of V. alginolyticus strain HY9901. Journal of Fish Diseases. 2022;**45**(3):421-434

[61] Chen C, Wang Q-b, Liu Z-H, et al. Characterization of role of the toxR gene in the physiology and pathogenicity of V. alginolyticus. Antonie Van Leeuwenhoek. 2012;**101**:281-288

[62] William HB, Robert AB, Hui W, et al. Oral biofilms: Pathogens, matrix, and polymicrobial interactions

in microenvironments. Trends in Microbiology. 2018;**26**(3):229-242

[63] Hider RC, Kong X. Chemistry and biology of siderophores. Natural Product Reports. 2010;**27**(5):637-657

[64] Schaible UE, Kaufmann SHE. Iron and microbial infection. Nature reviews. Microbiology. 2004;**2**(12):946-953

[65] Crosa JH, Walsh CT. Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiology and Molecular Biology Reviews: MMBR. 2002;**66**(2):223-249

[66] Challis GL. A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. Chembiochem: A European Journal of Chemical Biology. 2005;**6**(4):601-611

[67] Faraldo-Gómez JD, Sansom MSP. Acquisition of siderophores in gram-negative bacteria. Nature Reviews. Molecular Cell Biology. 2003;**4**(2):105-116

[68] Escolar L, Pérez-Martín J, de Lorenzo V. Opening the iron box: Transcriptional metalloregulation by the Fur protein. Journal of Bacteriology. 1999;**181**(20)

[69] CBiosca EG, Fouz B, Alcaide E, et al. Siderophore-mediated iron acquisition mechanisms in V. vulnificus biotype 2. Applied and Environmental Microbiology, 1996,**62**(3):928-935

[70] Christine J, Sebastian S. The role of motility as a virulence factor in bacteria. International Journal of Medical Microbiology. 2002;**291**(8):605-614

[71] Kazmierczak BI, Hendrixson DR. Spatial and numerical regulation of flagellar biosynthesis in polarly flagellated bacteria. Molecular Microbiology. 2013;**88**(4):655-663

[72] Atsumi T, Maekawa Y, Yamada T, et al. Effect of viscosity on swimming by the lateral and polar flagella of V. alginolyticus. Journal of Bacteriology. 1996;**178**(16):5024-5026

[73] Kawagishi I, Nakada M, Nishioka N, Homma M, et al. Cloning of a V. alginolyticus rpoN gene that is required for polar flagellar formation. Journal of Bacteriology. 1997;**179**(21):6851-6854

[74] Yang T, Qiyao W, Qin L, et al. Involvement of LuxS in the regulation of motility and flagella biogenesis in V. alginolyticus. Bioscience, Biotechnology, and Biochemistry. 2008;**72**(4):1063-1071

[75] Shota K, Michio H, Seiji K. Analysis of the GTPase motif of FlhF in the control of the number and location of polar flagella in V. alginolyticus. Biophysics and Physicobiology. 2017;**14**(0):173-181

[76] Correa NE, Peng F, Klose KE. Roles of the regulatory proteins FlhF and FlhG in the V. cholerae flagellar transcription hierarchy. Journal of bacteriology. 2005;**187**(18):6324-6332

[77] Kusumoto A, Kamisaka K, Yakushi T, et al. Regulation of polar flagellar number by the flhF and flhG genes in V. alginolyticus. Journal of biochemistry. 2006;**139**(1):113-121

[78] Balaban M, Hendrixson DR. Polar flagellar biosynthesis and a regulator of flagellar number influence spatial parameters of cell division in Campylobacter jejuni. PLoS pathogens. 2011;**7**(12):e1002420

[79] Pandza S, Baetens M, Park CH, et al. The G-protein FlhF has a role in polar

flagellar placement and general stress response induction in Pseudomonas putida. Molecular Microbiology. 2000;**36**(2):414-423

[80] Kusumoto A, Shinohara A, Terashima H, et al. Collaboration of FlhF and FlhG to regulate polar-flagella number and localization in V. alginolyticus. Microbiology (Reading, England). 2008;**154**(Pt 5):1390-1399

[81] Zhu S, Nishikino T, Hu B, et al. Molecular architecture of the sheathed polar flagellum in V. alginolyticus. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(41):10966-10971

[82] Shinoda S. Protein toxins produced by pathogenic vibrios. Journal of Natural Toxins. 1999;**8**(2):259-269

[83] Sigel SP, Payne SM. Effect of iron limitation on growth, siderophore production, and expression of outer membrane proteins of V. cholerae. Journal of Bacteriology. 1982;**150**(1):148-155

[84] Stoebner JA, Payne SM. Iron-regulated hemolysin production and utilization of heme and hemoglobin by V. cholerae. Infection and Immunity. 1988;**56**(11):2891-2895

[85] Dai JH, Lee YS, Wong HC. Effects of iron limitation on production of a siderophore, outer membrane proteins, and hemolysin and on hydrophobicity, cell adherence, and lethality for mice of Vibrio parahaemolyticus. Infection and Immunity. 1992;**60**(7):2952-2956

[86] Iida H. Hemolysins produced by vibrios. Journal of Toxicology: Toxin Reviews. 1997;**16**(4):215-227

[87] Zhang X, Austin B. Haemolysins in Vibrio species. Journal of Applied Microbiology. 2005;**98**(5):1011-1019

[88] Cai SH, Wu ZH, Jian JC, et al. Cloning and expression of gene encoding the thermostable direct hemolysin from V. alginolyticus strain HY9901, the causative agent of vibriosis of crimson snapper (Lutjanus erythopterus). Journal of Applied Microbiology. 2007;**103**(2):289-296

[89] Liu H, Xiao M, Zuo J, et al. Vanillic acid combats V. alginolyticus by cell membrane damage and biofilm reduction. Journal of Fish Diseases. 2021;**44**(11):1799-1809

[90] Hernández-Robles MF, Álvarez-Contreras AK, Juárez-García P, et al. Virulence factors and antimicrobial resistance in environmental strains of V. alginolyticus. International Microbiology. 2016;**19**(4):191-198

[91] Li L, Meng H, Gu D, et al. Molecular mechanisms of V. parahaemolyticus pathogenesis. Microbiological Research. 2019;**222**:43-51

[92] Morot A, El Fekih S, Bidault A, et al. Virulence of V. harveyi ORM4 towards the European abalone Haliotis tuberculata involves both quorum sensing and a type III secretion system. 2021;**23**:5273-5288

[93] Zhang, T. and M. Yang, Molecular mechanisms of virulence genes expression in V. parahaemolyticus. College of Animal Science and Technology. vol. 60(07). Zhejiang A&F University; 2020. pp. 1345-1357

[94] Cheng H. Function Study of the Virlence Genes acfA and toxR inV. alginolyticus. Guangdong Ocean University; 2014

[95] Quan T, Li W, Yang X. Investigation of the pathogenicity of V. alginolyticus isolated from patients with diarrhea. Chinese Journal of Public Health. 1985;**05**:15-18

[96] Suzuki M, Danilchanka O, Mekalanos JJ, V. cholerae T3SS effector VopE modulates mitochondrial dynamics and innate immune signaling by targeting Miro GTPases. Cell Host Microbe. 2014;**16**(5):581-591

[97] Roehrich AD et al. Steps for shigella gatekeeper protein MxiC function in hierarchical Type III secretion regulation. Journal of Biological Chemistry. 2017;**292**(5):1705-1723

[98] Pang H et al. Acetylome profiling of V. alginolyticus reveals its role in bacterial virulence. Journal of Proteomics. 2020;**211**:103543

[99] Mao F et al. Virulence of V. alginolyticus Accentuates Apoptosis and Immune Rigor in the Oyster Crassostrea hongkongensis. Frontiers in Immunology. 2021;**12**:746017

[100] Wang X, Quinn PJ. Lipopolysaccharide: Biosynthetic pathway and structure modification. Progress in Lipid Research. 2010;**49**(2):97-107

[101] Zhang G, Meredith TC, Kahne D. On the essentiality of lipopolysaccharide to Gram-negative bacteria. Current Opinion in Microbiology. 2013;**16**(6):779-785

[102] Maldonado RF, Sá-Correia I, Valvano MA. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiology Reviews. 2016;**40**(4):480-493

[103] Jian J, Ye J, Wu Z. Influence of lipopolysaccharide fromibrio alginolyticus on immunological function ofepinephelus skaara. Acta Hydrobiologica Sinica. 2004;**01**:103-105

[104] Chen Q, Yan Q, Ma S. Progress on pathogenicity research of V. alginolyticus. Marine Sciences. 2006;**08**:83-89

[105] Xu X et al. A modified silver-staining method for lipopolysaccharides detection. Biotechnology Bulletin. 2009;**03**:95-97

[106] Zhang J et al. Preparation of V. parahaemolyticus lipopolysaccharide and lmmunological analysis of the lipopolysaccharide against paralichtys olivaceus. Periodical of Ocean University of China. 2004;**04**:571-576

[107] Smith JA. Brucella Lipopolysaccharide and pathogenicity: The core of the matter. Virulence. 2018;**9**(1):379-382

[108] Zhou Q. Study of Lipid A Structure and Related Genes in V. parahaemolyticus. Southern Yangtze University; 2020