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Molecular mechanisms of *Vibrio parahaemolyticus* pathogenesis

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ABSTRACT

Vibrio parahaemolyticus is a Gram-negative halophilic bacterium that is mainly distributed in the seafood such as fish, shrimps and shellfish throughout the world. *V. parahaemolyticus* can cause diseases in marine aquaculture, leading to huge economic losses to the aquaculture industry. More importantly, it is also the leading cause of seafood-borne diarrheal disease in humans worldwide. With the development of animal model, next-generation sequencing as well as biochemical and cell biological technologies, deeper understanding of the virulence factors and pathogenic mechanisms of *V. parahaemolyticus* has been gained. As a globally transmitted pathogen, the pathogenicity of *V. parahaemolyticus* is closely related to a variety of virulence factors. This article comprehensively reviewed the molecular mechanisms of eight types of virulence factors: hemolysin, type III secretion system, type VI secretion system, adhesion factor, iron uptake system, lipopolysaccharide, protease and outer membrane proteins. This review comprehensively summarized our current understanding of the virulence factors in *V. parahaemolyticus*, which are potentially new targets for the development of therapeutic and preventive strategies.

1. Introduction

Vibrio parahaemolyticus is the leading cause of diarrheal disease associated with seafood worldwide. Symptoms after ingestion include two to ten days of watery diarrhea, abdominal cramps, nausea, vomiting, and headaches (DePaola et al., 2003; Austin B, 2010). Septicemia following gastroenteritis and wound infections can lead to mortality, particularly for individuals with preexisting liver disease. Antibiotics have been used as the main treatment for *V. parahaemolyticus* infection (Letchumanan et al., 2014; Letchumanan et al., 2016). However, antibiotics may interfere with beneficial microbiota and promote post-antibiotic pathogen expansion (Langdon et al., 2016; Laniro et al., 2016). More importantly, recent studies have shown that majority of *V. parahaemolyticus* isolated from both environmental and clinical settings are multi-drug resistant, highlighting that alternative treatment and prevention strategies are needed (Freire-Moran et al., 2011; Yano et al. (2013); Obaidat et al., 2017; Lee and Raghunath, 2018). Previous studies have shown that the bacteriophage could be used to control *V. parahaemolyticus* (Letchumanan et al., 2016; Zhang et al., 2018a). Targeting virulence determinants has been increasingly applied for the development of alternative antibacterial treatments. With the development of the first infant rabbit animal model that recapitulate many symptoms in humans, next generation sequencing and biochemical, cell biological technologies, significant advances have been made on the virulence factors and pathogenic mechanisms of *V. parahaemolyticus*. This review articles summarized eight types of virulence factors and the molecular mechanisms by which they contribute to the pathogenesis of *V. parahaemolyticus*.

2. Hemolysins

It is currently known that *V. parahaemolyticus* can produce three hemolysins: thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH) and thermolabile hemolysin (TLH) encoded by the *tdh*, *trh* and *tlh* genes, respectively. TDH and TRH are considered to be the main pathogenic factors (Baker-Austin et al., 2010; Mahoney et al., 2010; Paranjpye et al., 2012). Epidemiological studies showed that most of the clinical isolates contained *tdh* and *trh* genes, while very few environmental isolates contained *tdh* and *trh* genes (Theethakaew et al., 2013). However, recently studies also showed that *tdh* and *trh* were

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identified from 48% and 8.3% of the environmental isolates, respectively Gutierrez West et al. (2013), indicating that *tdh* and *trh* genes may also play a role in the bacterial fitness to the environment. TDH is a heat-resistant, pore-forming toxin composed of 156 amino acids (Sakurai et al., 1973; Hamada et al., 2007). Studies have shown that mannitol, high concentrations of sodium chloride, bile acids and amino acids can promote the production of TDH (Osawa et al., 2002). There is only one copy of the *tdh* gene in the tdh^+trh^+ strain. In contrast, $tdh2^+RH^+$ strain has two copies of *tdh*: *tdh1* and *tdh2*, both of which encode proteins with hemolytic function (Kishishita et al., 1992). However, *tdh2* has higher expression level than *tdh1* (Shalu et al., 2012). TDH acts directly on red blood cells and has hemolytic activity, enterotoxin activity, cardiotoxicity and cytotoxicity.

The hemolysis process of TDH is mediated by receptors. TDH tightly binds erythrocyte cell membranes and destroys cell membrane and lysosomal membrane in a temperature-dependent manner (Huntley et al., 1993). The transcriptional regulatory factor Hfq inhibits the expression of *tdh* gene (Nakano et al., 2008), while CalR inhibits *tdh2* transcription (Zhang et al., 2017a). The mechanism of cytotoxicity of TDH is different from its hemolytic activity (Naim et al., 2001). High concentration of TDH increases the concentration of calcium ions in intestinal epithelial cells, leading to the opening of the chloride channel, and increased secretion of chloride ions in the intestinal cells (Raimondi et al., 1995; Takahashi et al., 2000a, b; Takahashi et al., 2001). *trh* shares 54.8–68.8% homology with *tdh*, and is present in a small number of clinical isolates (Kishishita et al., 1992). TRH is composed of 189 amino acids, *trh1* and *trh2* are two encoding genes of TRH, and the similarity of *trh1* and *trh2* are 84% (Nishibuchi et al., 1989; Kishishita et al., 1992). Isolate carrying *trh2* does not contain the *tdh* and *trh1* genes, suggesting that *trh2* does not coexist with the *tdh* and *trh1* genes (Nishibuchi et al., 1989). TLH is an atypical phospholipase that can only exert hemolysis in the presence of lecithin (Zhang et al., 2005). Expression of *tlh* is up-regulated in the intestine, but the function and pathogenesis of TLH is still unclear (Gotoh et al., 2010; Broberg et al., 2011). Both clinical isolates and environmental isolates contain *tlh*. Due to the highly conserved of the TLH, and studies showed that *tlh* could be used for detection of *V. parahaemolyticus* (Park et al., 2013). The newly established method Propidium monoazide-Loop mediated isothermal amplification (PMA-LAMP) targeting *tlh*, *tdh* and *trh* gene can rapidly, sensitively and specifically screen the VBNC status of *V. parahaemolyticus* (Zhong et al., 2017). A green fluorescent protein-based TLH antibody has been developed, which provides the possibility to monitor TLH in real time (Chen et al., 2015). Overall, these studies suggested that TDH, TRH and TLH may play an important role in the pathogenesis of *V. parahaemolyticus* during infections in humans, and the combination of these genes could be potentially used for differentiating virulent strains from those that are less virulent.

The study showed that TDHs trigger NLRP3 inflammasome activation, while the activation of NLRP3 could promotes the elimination of pathogens (Higa et al., 2013). The TDHs may be used in the evasion of inflammasome sensing at the interaction between host and microbe. Besides, development of strategies that specifically targeting other hemolysins could be potentially used to reduce the virulence of *V. parahaemolyticus*.

3. Type III secretion system (T3SS)

T3SS is usually encoded by a 30–40 kb genetic region present either in a plasmid or chromosome as a pathogenicity island (PAI) (Calder et al., 2014a). T3SS is a major virulence factor, which can inject a variety of effector proteins into host cells through a syringe-like transmembrane device. Effectors manipulate modify host's protein function or signaling pathways to exert virulence (Shames and Finlay et al., 2012; Portaliou et al., 2016). T3SS consists of over 30 proteins, which can be divided into four categories: structural proteins, translocators, effector proteins and molecular chaperones (Zhou et al., 2013). *V. parahaemolyticus* has two sets of T3SSs, which are located on chromosome 1 (T3SS1) and chromosome 2 (T3SS2) (Calder et al., 2014a). T3SS1 mainly contributes to cytotoxicity, while T3SS2 mainly contributes to enterotoxicity (Hiyoshi et al., 2010). Both clinical and environmental isolates contain eight T3SS1 genes (VP1667, VP1690, VP1656, VP1680, VP1686, VP1696, VP1670 and vp1659) including the secretion apparatus, putative translocators or effectors, thus T3SS1 in combination with Kanagawa test (hemolysis test) can be used as a genetic marker for *V. parahaemolyticus* (Paranjpye et al., 2012). T3SS1 mainly affects the biofilm formation, motility and cytotoxicity, and contributes to the survival of *V. parahaemolyticus* in the environment. T3SS2 is involved in the negative regulation of cellular inflammatory response, which is conducive to the process of immune evasion of pathogenic bacteria in the host (Calder et al., 2014a). Analysis of the *V. parahaemolyticus* isolates obtained from the stool samples of 1900 diarrheal patients in Shanghai Hospital found that T3SS1 and T3SS2 were present in all isolates, and only the *trh*+ strain contained the T3SS2β gene (Li et al., 2017a).

Regulation of T3SS1 in *V. parahaemolyticus*. T3SS1 is regulated by a cascade of regulatory systems, which composes the interaction of EsxA, ExsC, ExsD and ExsE proteins (Fig. 1) (Kodama et al., 2010a). Transcription of T3SS1 is initiated by ExsA (Liu and Thomas et al, 2015), a transcription factor belonging to the AraC family. ExsD is an anti-activator that binds directly to ExsA and inhibits the activity of ExsA. ExsC is an anti-anti-activator that can directly bind ExsD to release ExsA (Zhou et al., 2008, 2010a). ExsE contributes to the negative regulation of T3SS1 and modulation of the adherence phenotype while infection the HeLa cells (Erwin et al., 2012). *in vitro* studies have shown that growth under DMEM condition or contact with epithelial cells, e.g., HeLa or Caco-2 cells, stimulates the expression of T3SS1 via modulating the expression level of ExsC, ExsA, ExsD and their interaction (Zhou et al., 2010b). *in vivo* transcriptome studies also showed that *V. parahaemolyticus* in the small intestine has higher level of T3SS1 transcription than those grown *in vitro* under LB condition (Zhou et al., 2010b). In addition, the HlyU and H-NS can modulate the T3SS1 through regulating the expression of *exsA* (Kodama et al., 2010a; Getz et al., 2018). Besides, the CalR and ToxR protein also involved in the regulation of T3SS1 (Osei-Adjei et al., 2017). Interestingly, recent studies demonstrated that bacterial colonization of *exsD* mutant is reduced comparing to the wild-type, suggesting that the overexpression of T3SS1 impairs bacterial colonization in the small intestine (Hubbard et al., 2016). Both the NLRP3 and NLRC4 inflammasomes are activated by T3SS1 in response to *V. parahaemolyticus* infection indicating that T3SS1 effectors appears to be one strategy for bacterial evasion of the host proinflammatory response (Higa et al., 2013). Thus, overexpression of T3SS1 may lead to high level of inflammatory response, which is harmful for bacterial colonization in the small intestine. It remains to be elucidated that what signals in the small intestine modulate T3SS1 expression and how overexpression of T3SS1 impairs its colonization.

Effectors of T3SS1. T3SS1 has four secreted proteins: VopQ, VopS, VPA0450 and VopR (VP1683). VopQ is the most important protein responsible for T3SS1-mediated eukaryotic cytotoxicity. VopQ binds to V-ATPase on lysosomal membrane and changes the proton concentration in the lysosome, which promotes lysosomal cleavage and autophagic vesicle formation, leading to host cell autophagy and cytotoxicity. VopQ can also promote IL-8 secretion by activating p38 in the MAPKs signaling pathway (Matlawska-Wasowska et al., 2010; Shimohata et al., 2011). VopS is a post-translational regulatory protein. It contains a Fic domain that mediates adenylation (AMPylation) of Rho GTPase (RhoA, Rac1 and Cdc42), resulting in its inability to bind to downstream substrate proteins and collapse of the actin cytoskeleton (Casselli et al., 2008).

VPA0450 is an inositol phosphatase that hydrolyzes the phosphate group of 4,5-diphosphophosphatidylinositol on the plasma membrane (Broberg et al., 2010). Subsequently, the connection between actin

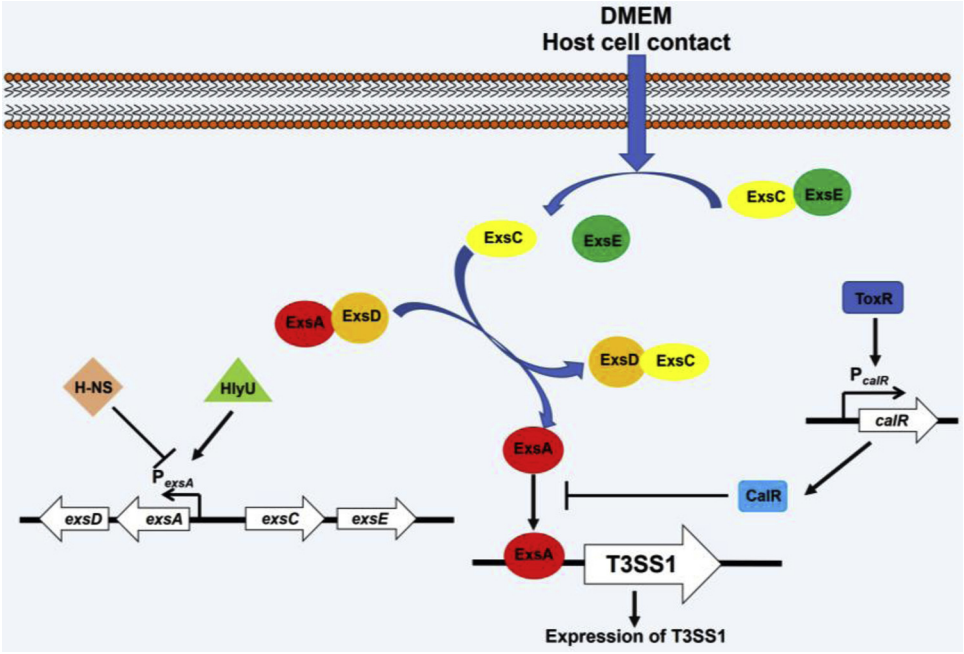

Fig. 1. Regulation of T3SS1 genes in *V. parahaemolyticus*. When culture at DMEM medium or contact the host cell, the ExsC binds ExsD and release the ExsA, then the ExsA protein can directly promote the expression of T3SS1. Besides, the HlyU and H-NS can regulate the expression of *exsA*, while the CalR can inhibit the ExsA to bind T3SS1 promoter.

cytoskeleton and the plasma membrane is disrupted, leading to the formation of bubble in the cytoplasmic membrane, cell rounding and lysis (Broberg et al., 2010). VopR is encoded by locus vp1683 and contains a domain similar to the phosphoinositide domain (BPD). N-terminal domain of VopR binds to phosphoinositide on the host cell membrane. VopR may promote the correct folding of T3SS effectors in the host cells (Broberg et al., 2010; Salomon et al., 2013a). The correct secretion of many effectors requires molecular chaperones. There are currently two T3SS1 specific chaperone proteins: VecA and VPA0451. VecA is a chaperone protein of VopQ. VecA can direct bind to VopQ, which is essential for its secretion and stability in the host cells (Akeda et al., 2009). VPA0451 is a chaperone protein of VPA0450 and required for VPA0450 translocation into the host cell membrane (Waddell et al., 2014).

Regulation of T3SS2 in *Vibrio parahaemolyticus.* T3SS2 is only present in the pathogenic *V. parahaemolyticus* isolated from patients with sepsis and other diseases and has a low similarity with other T3SS. Therefore, T3SS2 is a specific secretion system for pathogenic *V. parahaemolyticus* (Li et al., 2017b). The expression of T3SS2 is regulated by two transcriptional regulators (VtrA and VtrB). VtrA acts as a transmembrane protein and upregulates the expression of *vtrB*, and VtrB in turn activates the transcriptional expression of T3SS2 genes. VtrA and VtrC form a barrel-like structure on the cell membrane of *V. parahaemolyticus*. Such barrel-like structure acts as a receptor that can recognize and bind bile salt, leading to VtrB and subsequent T3SS2 gene expression (Kodama et al., 2010b; Li et al., 2016). ToxR protein plays essential roles in the pathogenicity of *V. parahaemolyticus* and contributes to the colonization of *V. parahaemolyticus* in the mammalian intestine (Hubbard et al., 2016). Studies showed that the overexpression of VtrA in the absence of ToxR can restore the expression of T3SS2 (Hubbard et al., 2016), indicating that ToxR controls the expression of T3SS2 through VtrA. It is possible that ToxR enhances the ability of VtrA or the protein-protein interactions between ToxR and VtrA, leading to the activation of VtrB expression and further increase the expression of T3SS2 (Fig. 2).

Effectors of T3SS2. The enterotoxicity of T3SS2 is mediated its effectors that destroy the cytoskeleton and manipulate cell signaling transduction. Currently, seven effectors of T3SS2 (VopA, VopT, VopL, VopV, VopC, VopZ and VPA1380) have been identified and characterized (Fig. 3) (Trosky et al., 2004; Kodama et al., 2007; Liverman et al., 2007; Hiyoshi et al., 2011; Zhang et al., 2012; Zhou et al., 2013; Calder et al., 2014b). VopA and VopZ inhibit immune response by inhibiting different components of the MAPKs signaling pathway (Trosky et al., 2004). VopC, VopL and VopV can act on the actin cytoskeleton in the host cells. VopA is an acetyltransferase that acetylates Ser/Thr residues of MAPKs and blocks MAPKs signaling, thereby inhibiting cell growth and ultimately leading to cell death (Trosky et al., 2004). VopZ inhibits the activation of kinase (TAK1), which in turn inhibits the downstream kinases MAPK and NF-κB signaling pathways (Zhou et al., 2013). It is hypothesized that inhibition of TAK1 activation may lead to disruption of the intestinal epithelial cells and diarrheal disease as deletion of TAK1 can cause intestinal epithelial cell damage, death, and inflammatory response (Zhou et al., 2013). VopT is an ADP ribosyltransferase that modifies Ras GTPase by transferring ADP-ribose from NAD + to the substrates. VopT is one of the effectors responsible for T3SS2-mediated cytotoxicity (Kodama et al., 2007). VopC has deamidase/transglutaminase activity. VopC activates Rac1 and Cdc42 by deamidation, leading to the modification of actin cytoskeleton. Therefore, VopC can induce changes in actin cytoskeleton and promote *V. parahaemolyticus* invasion into host cells (Zhang et al., 2012). VopV is a key effector that can bind both actin and filamin with its repeat and C-terminus domains, respectively. More importantly, binding to either actin or filamin is sufficient to mediate its diarrheal disease, suggesting these two actin and filamin binding domains are functionally redundant (Hiyoshi et al., 2011). It remains to be elucidated how binding of VopV to either actin or filamin leads to diarrheal disease. VopL binds to microfilaments to replace nucleation promoting during in microfilament synthesis, leading to the acceleration of nucleation reactions (Liverman et al., 2007). VopL can also neutralize reactive oxygen species (ROS) produced by the nicotinamide dinucleotide phosphate (NADPH) oxidase complex in host cells to facilitate bacteria survival in host cells (de Souza Santos et al., 2017). VPA1380 has a structure similar to the cysteine protease domain in various bacterial toxins and requires inositol hexose phosphate (IP6) as an activator to mediate cytotoxicity (Calder et al., 2014b).

4. Type 6 secretion system (T6SS)

Structure and function of T6SS in *V. parahaemolyticus*. T6SS is also a secretory device capable of secreting effectors into host mammalian

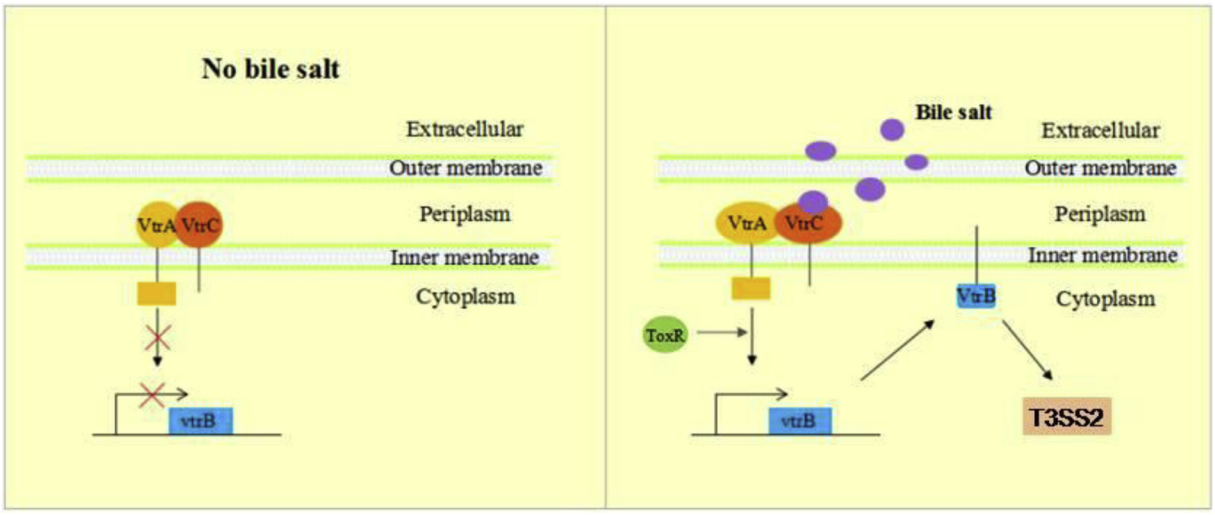

Fig. 2. Regulation of T3SS2 in *V. parhaemolyticus*. While there is no bile salt, VtrC binds VtrA, and inhibits expression of *vtrB*. When bile salt is present, it binds VtrC and releases VtrA protein, leading to the expression of *vtrB*, and T3SS2. ToxR regulates the ability of VtrA to activate the expression of *vtrB*.

cells as well as target bacterial cells. Structurally, T6SS is similar T1SS, T3SS and T4SS (Fig. 4). In *V. parahaemolyticus*, there are two T6SSs (T6SS1 and T6SS2 located on chromosome 1 and 2, respectively). Both T6SS1 and T6SS2 can be present in clinical and environmental isolates (Li et al., 2017a). T6SS2 is expressed and is most active under low salt conditions (Salomon et al., 2013b). T6SS consists of a series of components including structural proteins, translocators, secreted proteins, and some other proteins with auxiliary function (Bönemann et al., 2010). The core components of T6SS structural proteins, DotU and IcmF, are located in the cell membrane and stabilize the stability of the plasma membrane (Bönemann et al., 2010). The ClpV ATPase provides energy for the secretory function of T6SS. Typically, ClpV interacts with IcmF to initiate an energy supply system. The T6SS translocation protein Hcp (hemolysin co-regulatory protein) and VgrG (valine-glycine repeat protein G) together constitute a tube structure to secrete proteins, which Hcp is a six-membered ring protein that can be stacked in the periplasm to form a long tube that transports secreted proteins (Sha et al., 2013). The VgrG protein forms a needle-like spike structure that penetrates the lipoprotein bilayer of the cell membrane and exposes the hidden effector region within the host cell membrane to the cytoplasm. VgrG binds to the target protein in the host cell, inducing physiological response. When the VgrG is detached from Hcp tube, the secreted protein of T6SS is released into the host cell through the Hcp tube (Durand et al., 2014). The T6SS2 in *V. parahaemolyticus* plays a role in bacterial invasion into host cells. VgrG2 of T3SS2 can also induce autophagy in macrophage RAW264.7 (Yu et al., 2015). STK (a serine/threonine kinase) and STP (a serine/threonine phosphatase) regulate the biological activity of T6SS by phosphorylation and dephosphorylation of Fha (Mougous et al., 2007).

Regulation of T6SS. T6SS in *V. parahaemolyticus* is regulated by quorum sensing (QS) system. OpaR and AphA are the core regulators of QS that are highly expressed at high bacterial density and low bacterial

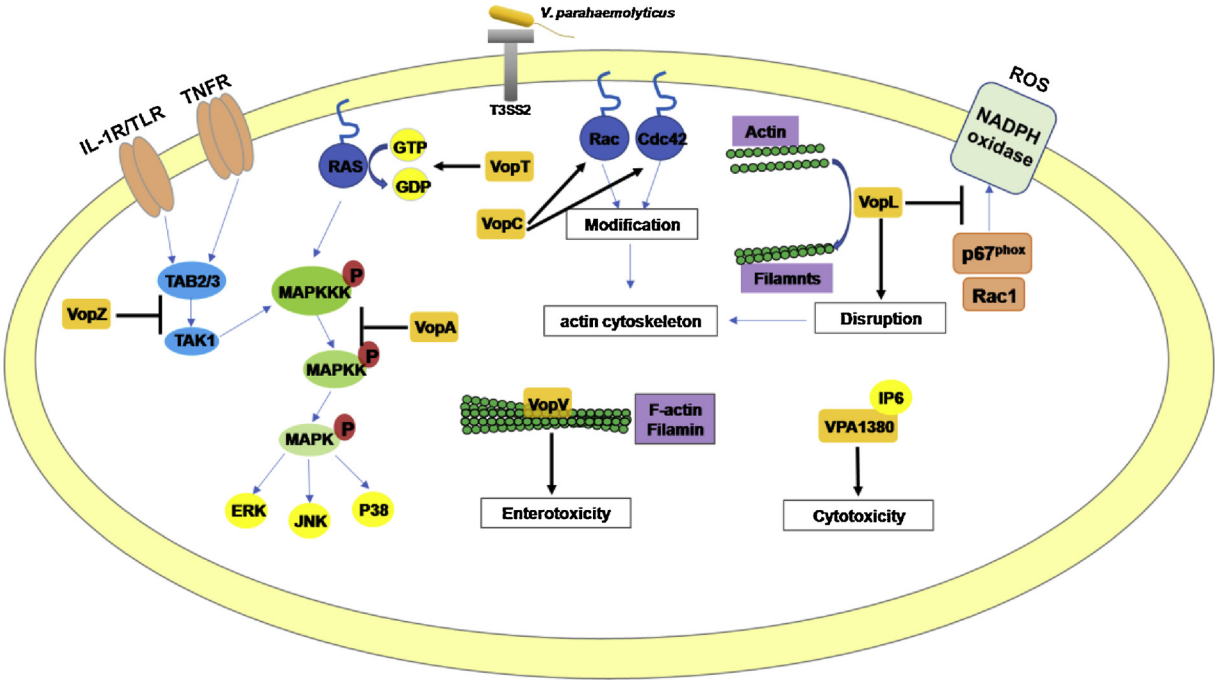

Fig. 3. Function of T3SS2 effectors. Seven effectors of T3SS2 (VopA, VopV, VopL, VopT, VopC, VopZ, VPA1380) have been characterized. The signaling pathways that are hijacked or impaired by these effectors are shown.

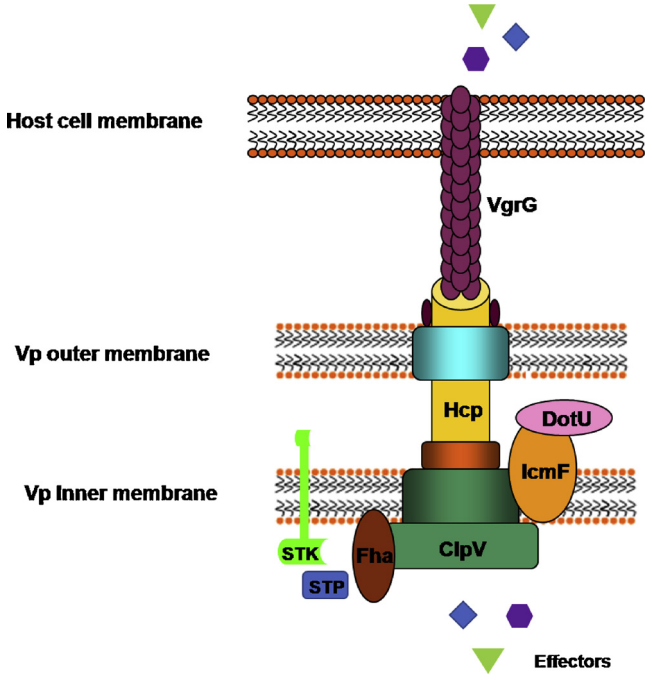

Fig. 4. Structure of Type VI secretion system. IcmF and DotU is the core element of T6SS and located at the inner membrane to stabilize the stability of the plasma membrane. ClpV interacts with the IcmF to provide the energy, and the STK can regulate the T6SS by phosphorylation and dephosphorylation of Fha. VgrG can be secreted to the host cell through the Hcp tube.

density, respectively. At low cell densities, the regulatory factor LuxO is active, inhibiting the activity of OpaR and activating AphA. At high cell densities, the activity of LuxO is restricted, resulting in inhibition of AphA activity and activation of OpaR (Kalburge et al. (2017)). OpaR can inhibit the transcription of hcp1 and negatively regulate T6SS1, but positively regulate T6SS2 (Ma et al., 2012). AphA has a negative regulatory effect on both T6SS1 and T6SS2 (Wang et al., 2013; Zhang et al., 2017c). H-NS is a nucleic acid binding protein and can bind to AT-rich DNA sequences, thereby inhibiting gene transcription. H-NS inhibits the expression of both T6SS1 and T6SS2 (Salomon et al., 2014; Sun et al., 2014). CalR (a member of LysR family) can positively regulate the expression of T6SS1 and T6SS2. CalR is an antagonistic factor of H-NS and can activate the transcription of the T6SS2 gene by competing for the binding site of the H-NS to the promoter region of the target genes (Zhang et al., 2017b). As a membrane-bound virulence-regulating protein of *V. parahaemolyticus*, ToxR works together with AphA and OpaR to inhibit the activity of T6SS1. The expression of *toxR* gene is closely related to QS. In addition, T6SS1 has the highest expression level between when OD600 is between 0.6 and 0.8, indicating that the virulence of *V. parahaemolyticus* is inextricably linked with the bacterial QS (Zhang et al., 2017c).

5. Adhesion factors

The adhesion of pathogens is closely related to their pathogenicity. Microbial adhesion to host tissues is the initial event during the process of infection (Navarre et al., 1999). The main factors that are associated with *V. parahaemolyticus* adhesion to host cells are hemagglutinin, enolase and T6SS2 (Yamamoto et al., 1989; Jiang et al., 2014; Zhang et al., 2017). Studies have shown that mannose-sensitive hemagglutinin (MSHA) is an important factor affecting bacterial-host cell adhesion and pathogenicity. MSHA has a high affinity for polysaccharides such as sialic acid and GM1 ganglioside, indicating that MSHA might be receptors for MSHA in the gut, promoting their colonization to intestinal epithelial cells (O'Boyle et al. (2013)). In *V. parahaemolyitucs*, the enolase is a plasminogen-binding, surface-exposed protein. Enolase could facilitate bacterial adherence to Hep-2 cells, which could promote pathogen-host interaction (Jiang et al., 2014). Besides, the CalR could regulate the *V. parahaemolyticus* adhesion by regulating the expression of T6SS2 (Zhang et al., 2017). The sigma factor RpoN is a regulatory protein that controls the synthesis of flagella. The *rpoN* mutant has a higher colonization in the intestine than the wild-type, indicating that RpoN may significantly affect the colonization of *V. parahaemolyticus* (Whitaker et al., 2014). The adhesion factor MAM-7, which is widely distributed in Gram-negative bacteria, binds to fibronectin and membrane phosphatidic acid on the host cell membrane to assemble a three-molecular polymer, which mediates pathogen adhesion to host cells. At the initial stage, the expression of MAM-7 in non-pathogenic lactic acid bacteria can make them adhere to host cells (Beltran et al., 2016). Inhibition of MAM-7 expression can reduce the adhesion and virulence of *V. parahaemolyticus* (Krachler et al., 2011). This phenomenon is also present in other Gram-negative bacteria containing MAM-7, suggesting that MAM-7 is a good vaccine or therapeutic target to prevent and treat infections caused by Gram-negative bacteria. Deeper comprehensive understanding of the adhesion mechanism could provide the new perspective to control the colonization of *V. parahaemolyticus* in the host and interrupt the first step of infection.

6. Iron uptake system

A recent study found that metal ions play an important role in the regulation of gene expression in *V. parahaemolyticus*. High calcium and low iron growth conditions stimulate group behavior and T3SS transcription (Gode-Potratz et al., 2010). Iron is an indispensable element in the growth and metabolism of some pathogens. The iron in the host cells is mainly found in red blood cells, lactoferrin and transferrin, and there are very few free iron ions, so it cannot meet the demand of iron for pathogens. There are two main ways for pathogenic Vibrio to obtain iron: one is to produce exotoxin to destroy red blood cells and release hemoglobin; the other is to produce a low molecular weight iron chelating agent, which has high affinity for iron ions in heme (Yamamoto et al., 1994; Kustusch et al., 2011). The iron uptake system in *V. parahaemolyticus* consists of an iron membrane-bound receptor and an iron chelate. *V. parahaemolyticus* can produce a carrier, called vibrioferrin, capable of chelation of irons, and the resulting iron-carrier complex can be transported to cells via the bacterial outer membrane protein receptor for iron assimilation (Yamamoto et al., 1995). Under the iron sufficient condition, Fur, as a DNA-binding protein, can inhibit the transcription of genes associated with the iron uptake system (Tanabe et al., 2013). Studies have shown that iron overload in humans increases the virulence of pathogens (Bullen et al., 1991; Berlutti et al., 2005; Pieracci et al., 2005). In humans, iron is found mainly in hemoglobin (Hb), heme, hemin, and iron chelators (Tf, Lf, or Ft). As an iron carrier, vibrioferrin binds to the iron-binding protein of the host cell and is recognized by the receptors PvuA1 and PvuA2 on the outer membrane of *V. parahaemolyticus* cells (Tanabe et al., 2003). Heme and ferritin (Hb) can also be recognized by these receptors. PvuA can bind the ABC transport system, the PvuBCDE system in the inner membrane, which transports iron-Vibrioferrin to the inner membrane (Tanabe et al., 2011). In addition, *V. parahaemolyticus* also contains the TonB system, which is composed of TonB, TonB1 and TonB2. Iron-Vibrioferrin is transported to the inner membrane by PvuA1 by the energy supplied by TonB1. Energy supply of PvuA2 system depends on TonB1 and TonB2 (Kuehl et al., 2010). *V. parahaemolyticus* can also utilize siderophores produced by other bacteria, such as exogenous aerobactin, desferri-ferrichrome, and enterrobactin (Funahashi et al., 2003, 2009; Tanabe et al., 2012).

V. parahaemolyticus can secrete an iron-reducing protein, which reduces the ferric ion to divalent iron. Besides, *V. parahaemolyticus* can also degrade hemoglobin (Hb) by secreting a protease to obtain iron from the humans (León-Sicairos et al., 2015). In addition, some outer

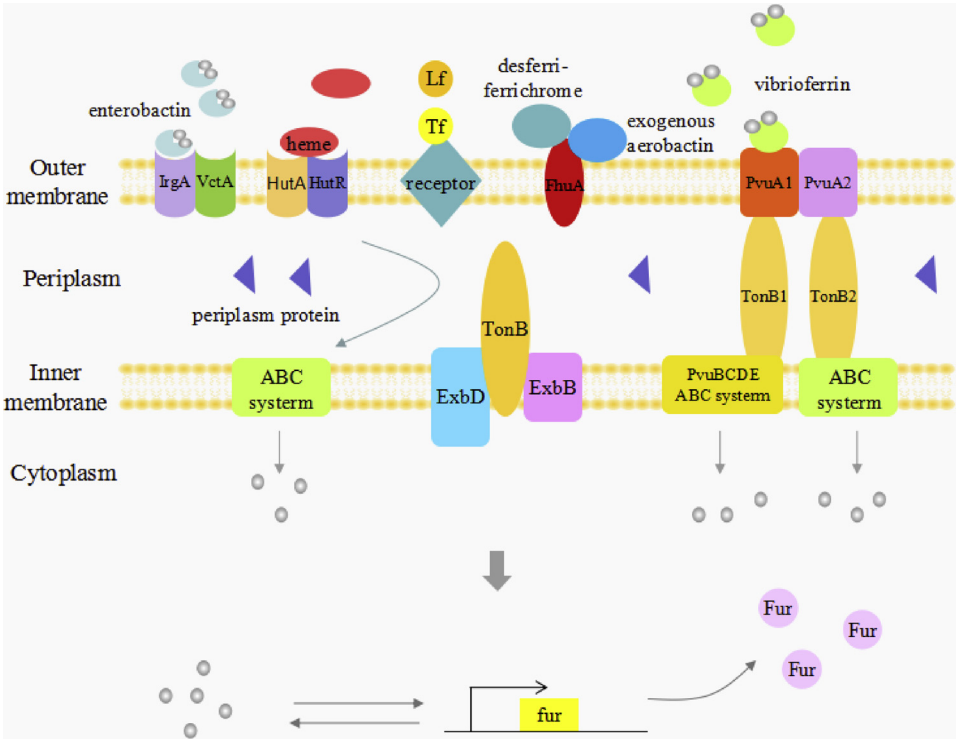

Fig. 5. The molecular mechanism of iron uptake system. The iron uptake system contains an iron membrane-bound receptor and an iron chelate in the outer membrane. Iron-Vibrioferrin can be transported to the inner membrane by PvuBCDE system, TonB system or ABC system. The DNA-binding protein Fur can regulate the expression of genes associated with the iron uptake system.

membrane protein receptors are important for the acquisition of iron. VctA and IrgA are receptors for enterrobactin, FhuA is a receptor for desferrin-ferrichrome and aerobactin, and HutA and HutR are receptors for heme (Tanabe et al., 2012). This outer membrane protein receptor transports the iron-iron carrier into the periplasm and binds to the periplasmic binding protein. This process requires TonB, ExbB, and ExbD on the inner membrane to provide energy and ultimately transport it to the interior of the cell via the ABC system (Fig. 5) (O'Malley et al. (1999); Kustusch et al., 2011; Tanabe et al., 2011, 2012).

7. Lipopolysaccharide (LPS)

LPS is the main substance of bacterial endotoxin, and one of the main components of the cell wall in Gram-negative bacteria. LPS consists of three parts: core polysaccharide, lipid A and O antigen. The formation of extracellular polysaccharide (EPS) in biofilm plays a crucial role in bacterial adhesion to living organisms and non-living organisms. Transcription and translation of cpsA-J operon are controlled by ScrABC, extracellular c-di-GMP concentration, CpsS-CpsR-CpsQ regulator and quorum sensing. H-NS can positively regulate the transcription of cpsA-J to promote EPS production (Zhang et al., 2018b). Bandekar et al. have shown that LPS of *V. parahaemolyticus* has an effect on murine peritoneal macrophages (Bandekar and Nerkar (1988)). By increasing the dose of LPS, RNA content and lysosomal activity of peritoneal macrophages can be significantly increased. Studies have found that the recombinant protein LVLGBP can be used as a pattern recognition protein (PRP) to recognize and bind LPS and activate innate immune response in shrimp (Valli et al., 2012; Chen et al., 2016).

8. Proteases

Protease secreted to the outside the cell is closely related to the pathogenesis of Vibrio. For example, in *Vibrio cholerae*, hemagglutinin protease can not only activate the A subunit of cholera enterotoxin, but also separate the bacteria from the host cell membrane and infect other host cells (Finkelstein et al., 1992). The *V. parahaemolyticus* strain also secretes hemagglutinin protease, but its mechanism of action is unclear. Lee et al obtained a protease A from a *V. parahaemolyticus* clinical isolate that does not contain *tdh* and *trh* genes and found that purified protease A has obvious toxic effects on experimental cells and has lytic activity on red blood cells (Lee et al., 2002). It can also cause tissue hemolysis, and death in mice in severe cases. Thus, protease A is a virulence factor of *V. parahaemolyticus*. Kim et al. cloned and expressed the metalloproteinase gene in *V. parahaemolyticus* and found that this protease has high homology with the collagenase of *Vibrio alginolyticus* (Kim et al., 2002). Since *V. alginolyticus* collagenase plays a role in infection, it is hypothesized that metalloproteinase may have a similar effect in *V. parahaemolyticus* infection. In addition, studies have found that in addition to metalloproteinases, extracellular serine proteases contribute to the pathogenicity of *Vibrio vulnificus* (Wang et al., 2008). In *V. parahaemolyticus*, PrtA acts as a serine protease with hemolytic activity and cytotoxicity (Osei-Adjei et al., 2018). The quorum sensing regulator (OpaR) and general stress response sensor (RpoS) in *V. parahaemolyticus* can regulate PrtA synthesis pathway by binding to the promoter of prtA gene. Both OprA and RpoS can up-regulate PrtA (Chang et al., 2018).

9. Outer membrane proteins (OMPs)

Vibrio OMPs are located at the outer membrane surface and thus can interact extensively with the outside of the cell (Li et al., 2014). LptD is an important outer membrane protein that plays an important role in mediating the transport of LPS to the outer leaflet. Studies have shown that LptD is widely distributed, protective, and has surface antigenic activity in the major Vibrio genus, indicating that LptD is involved in immune responses and has a strong ability to stimulate antibody responses (Zha et al., 2016). OMP is considered to be a potential candidate vaccine. To facilitate the screening and identification of potential candidate vaccines, immunoproteomics techniques based on two-dimensional electrophoresis and immunoblotting can be used to screen for immunogenic OMP (Pan et al., 2016).

10. Conclusion and prospective

As a halophilic food-borne pathogen, *V. parahaemolyticus* have various virulence factors responsible for hemolytic activity, cytotoxicity, intestinal toxicity, gastroenteritis, diarrhea, sepsis and even death. At present, the main virulence factors include hemolytic toxin, adhesin, protease, outer membrane protein, lipopolysaccharide, urease, type III (T3SS) and type VI (T6SS) secretion systems. In addition, the pathogenicity of *V. parahaemolyticus* is the result of a combination of multiple virulence factors, and thus it is necessary to comprehensively consider various virulence factors and the regulatory networks. Further studies on the mechanisms by which the environmental signals integrate into the genetic circuit to regulate the expression of these virulence factors would provide novel strategies to prevent and treat infection caused by *V. parahaemolyticus*.

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