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Genome sequencing of seafood-borne *Vibrio parahaemolyticus* VP49 reveals the presence of novel virulence attributes

BK Kumar* VK Deekshit, P Rai, I Karunasagar

Centre for Science Education and Research, Nitte University, Mangalore-575018, India

Abstract

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium autochthonous to the marine environments and responsible for seafood-borne gastroenteritis. Though the hemolysins TDH and/or TRH are classical virulence factors, several other potential virulence factors may contribute to their pathogenicity. In this study, *V. parahaemolyticus* was isolated from the seafood harvested along southwest coast of India and confirmed by standard biochemical and molecular methods. Genome sequencing of *V. parahaemolyticus* revealed the presence of T3SS2 operon in an approximately 44 kb region in close proximity to the hemolysin gene *trh*. The annotation of T3SS2 operon revealed the presence of genes encoding apparatus proteins *VscC2/R2/S2/T2/U2/N2*, *VcrD2*, an ATPase *VscN2*, translocons*VopB2/D2*, and effectors *VopA*/C/*L*. To the best of knowledge, this is the first report on sequencing and characterization of a T3SS gene cluster in seafood isolate of *V. parahaemolyticus* and this information will be of assistance in future studies to determine the different virulence attributes as well as mechanisms that enhance environmental or host fitness of *V. parahaemolyticus.* The presence of such virulence attributes in *V. parahaemolyticus* isolated from seafood suggests the potential of these isolates to cause infection in humans upon ingestion of contaminated seafood and questions the safety of seafood to consumers.

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Keywords:Vibrio parahaemolyticus; Virulence; Secretion system; T3SS2, Cytotoxicity

Corresponding author.E-mail address: krishnakumarb@nitte.edu.in

1. Introduction

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium autochthonous to the marine environments and is responsible for causing seafood-borne gastroenteritis in humans. The most of the disease causing strains isolated from the clinical cases are associated with the production of thermostable direct hemolysin (TDH) and TDH-related hemolysin coded by *tdh* and *trh* gene respectively. Majority of the strains isolated from the seafood and marine

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environments lacks gene coding for TDH and TRH, which is a characteristic that is observed in non-pathogenic/non-virulent strains. The presence of *V. parahaemolyticus* in seafood harvested along southwest coast of India has been well documented[1,2,3]. Another study highlightedthe prevalence (59%) of *trh*$^+$ isolates among the total *V. parahaemolyticus* isolated from seafood harvested along the Mangalore coast, India[2]. It is also observed that *V. parahaemolyticus* not only responsible for causing illness in human but also certain human pathogenic strains induces increased invasiveness and virulence capabilities in aquatic animals leading production losses[4]. *In vitro* cytotoxicity experiments conducted using *V. parahaemolyticus* isolated seafood showed that virulence determinants vary from strain to strain and their complex genome flexibility may affect their fitness to the host and their infection capabilities. In order to decode the genome of *V. parahaemolyticus* strains isolated from seafood were subjected to whole genome sequencing to identify virulence factors.

2. Materials and Methods

V. parahaemolytcus isolate (VP49) used in this study was isolated from the seafood harvested along southwest coast of India during the period April 2003–March 2013 and had been identified previously using standard bacteriological methodsand confirmed by PCR targeting species-specific marker*toxR* gene. Genomic DNA extracted from the *V. parahaemolyticus,* VP-49 using QIAamp DNA mini kit (Qiagen, Germany) was subject to genome sequencing on Ion Torrent PGM platform. The sequence data were assembled using CLC Genomics Workbench version 6.Structural gene prediction and functional annotation was performed using Rapid Annotations using Subsystems Technology (RAST) server

3. Results and Discussion

After the initial phenotypic and molecular investigation, genome sequencing of *V. parahaemolyticus* isolate VP49 was carried out to obtain the complete ORFs and genetic organizations of the T3SS2 operon .A total number of 1,017,077 reads with mean read length of 160 bp for 200 bp fragmentation chemistry obtained from the Ion PGM were assembled into 137 contigs. The draft genome had a length of 5047822bp as expected for *V. parahaemolyticus* with a total of 4,643 genes. The 137 contigs from VP-49 was also assembled to UCM-V493 (environmental isolate negative for *tdh* and *trh* and lacks all 7 pathogenicity islands). The gene coding for T3SS2 operon located in an approximately 44 kb region in the draft genome sequence of *V. parahaemolyticus* VP49. The linear maps of the T3SS regions were generated using Easyfig 2.1 (Fig. 1).

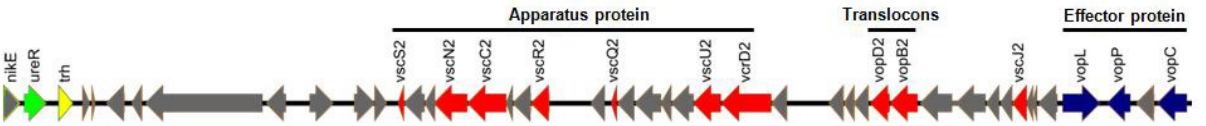

Fig. 1.Gene organization of approximately 44 Kb genomic regions consisting of T3SS2 genes in *V. parahaemolyticus*VP49.The arrows in red indicate the genes encoding putative apparatus proteins of T3SS and blue arrows the genes encoding putative regulatory and effector proteins of T3SS, and grey arrows indicate the genes encoding hypothetical proteins.

The T3SS2 operon of VP49 has a G+C content of 38.2%, which is considerably lower than the rest of the genome. Previous studies have demonstrated that, most of the T3SS2 genes has lower G+C, which is the characteristic of pathogenecity islands which are acquired through horizontal gene transfer. The annotation of the whole genome sequencing revealed that, the ORFs present in the T3SS2 of VP49 can be predicted to encode for apparatus proteins *VscC2/R2/S2/T2/U2/N2*, *VcrD2*, an ATPase *VscN2*, translocons*VopB2/D2*, and effectors *VopA*/C/*L*. All of the T3SS genes identified in *V. parahaemolyticus* VP49 were compared with the related genesthat exist in NCBI database and these showed high degree of similarity to that of *V. parahaemolyticus* Strain TH3996 isolated from aclinical sample.Phylogenetic analysis showed that T3SS2 identified in this study closely belongs to the T3SS2β found in the clinical isolates of *V. parahaemolyticus* TH3996, AQ4037 and *V. cholerae*. The gene coding *VopA* shared similarity with the gene coding for acteyltranferase group found in *V. parahaemolyticus* RIMD 2210633, *V. cholerae*, *Yersinia* spp. and *Aeromonas* spp with the presence of conserved residues such as histidine and glutamic acid. The protein

belongs to acteyltranferase group known to have a very potent kinase signalling inhibition, because of their capacity to do acetylation of the critical amino acid residues in the activation loop of MAPK kinases (MKKs) thereby blocking their activity by phosphorylation[5]. Hence we speculate that, in the marine ecosystem the *trh*$^+$ *V. Parahaemolyticus* might utilize the same pathway to overcome the defence mechanism of shellfish while existing as a commensal in the shellfish host and this may be the reason for the higher prevalence of *trh*$^+$ *V. parahaemolyticus* in seafood harvested from the tropical marine environment.Similarly, the gene coding for *VopC* protein identified in this study, showed sequence similarity to the catalytic domain of cytotoxic necrotizing factor (CNF) toxins with presence of catalytic cysteine (225th) and histidine (240th) residues in the core region. The T3SS2 effector proteins are known to be involved in the intracellular survival, replication, and pathogenesis of the organism.

In conclusion, the presence of the T3SS2 in *V. Parahaemolyticus* isolated from seafood along with hemolysin genes suggests the potential of these isolates to cause infection in humans upon ingestion of contaminated seafood. Further these isolates contain a group of virulence genes that may possibly disseminate in the marine ecosystem and hence they may also serve as progenitors for the other disease causing organisms by lateral transfer of virulence genes.To the best of knowledge, this is the first report on sequencing and characterization of a T3SS gene cluster in seafood isolate of *V. parahaemolyticus* and this information will be of assistance in future studies to determine the different virulence attributes as well as mechanisms that enhance environmental or host fitness of *V. parahaemolyticus.*

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