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**Genomic analysis of phage and *Vibrio parahaemolyticus***

**and evaluation of their therapeutic effects**

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**Abstract:**

**Background/Objective:** *Vibrio parahaemolyticus* (*V. parahaemolyticus*) is a ubiquitous pathogen found in various aquatic environments, including estuaries, seabed sediments, coasts, seawater, and marine animals. This bacterium causes significant diseases in aquatic animals, such as shrimp and crab acute hepatopancreatic necrotic disease (AHPND), *Penaeus vannamei*, and "glass seedling." As a result, it has become a major constraint on the development of the aquaculture industry and poses a serious threat to public health and safety. Traditional treatments for *V. parahaemolyticus* infections involve the use of antibiotics, but the misuse and overuse of these drugs have led to the emergence of multidrug-resistant bacteria, making prevention and treatment challenging. Recently, phages have gained attention as potential alternatives to antibiotics in treating bacterial diseases.

**Methods:** This study aimed to investigate the prevalence of *V. parahaemolyticus* in eastern coastal areas in China, analyze the virulence genes and drug resistance of clinical isolates, and compare the genomic information of clinical isolates. Additionally, we isolated virulent phage from *V. parahaemolyticus* and performed biological characterization, in vitro bacteriostatic capacity, biofilm removal, and comparative genomics studies. An experimental animal model for *Cherax quadricarinatus* (*C. quadricarinatus*) infection using *V. parahaemolyticus* LCU-VP2 (*pirA+* and *pirB+*) was established to evaluate the therapeutic effects of single phage, phage cocktail (vB\_VpaS\_SD2 and vB\_VpaS\_SD15), and antibiotic (florfenicol).

**Results:** From April 2022 to February 2023, we collected 282 samples from the east coast of China and found a *Vibrio* positivity rate of 79.79% (225/282). Upon isolation, 356 *Vibrio* strains were identified, with 132 being *V. parahaemolyticus* strains. The *V. parahaemolyticus* strains displayed high levels of resistance to β-lactams (97.7%, 129/132) and macrolides (60.6%, 80/132). A genome-wide analysis was conducted on 52 *V. parahaemolyticus* strains isolated from diseased *Penaeus vannamei* in aquaculture across four provinces in China (Guangdong, Jiangsu, Shandong, and Fujian) between 2019 and 2023. All of them carried more than 142 virulence genes. A total of 38 STs were identified, including 7 new STs with regional variations. The genomes of 169 *V. parahaemolyticus* strains were found to have open pan-genomes, and the core genome constructed phylogenetic trees with higher resolution than the MLST phylogeny. Fifteen *V. parahaemolyticus* phages were isolated and screened from 29 cultured water samples collected in Shandong Province. The total lysis rate reached 88.6% (117/132), with the highest lysis rate of a single phage at 48.48% (64/132). Biological characterization of long-tailed phages vB\_VpaS\_SD2 and vB\_VpaS\_SD15 revealed an optimal infection multiplicity of 0.1 and 1, respectively. One-step growth curves indicated an incubation period of 10 minutes and 20 minutes, with outbreak volumes of 16 PFU/mL and 23 PFU/mL, respectively, under conditions ranging from 4 to 45℃ and PH levels of 4 and 12. Both phage strains exhibited stability and activity within the same temperature and pH range, achieving a maximum lysis rate of 48% (64/132) for a single strain. Moreover, under conditions of 4 to 45℃ and pH levels of 4 and 12, these two phages demonstrated consistent high activity and significant in vitro bacteriostatic ability, along with effective biofilm removal. Whole-genome sequencing revealed that vB\_VpaS\_SD2 and vB\_VpaS\_SD15 are double-stranded DNAs of lengths 76,828 bp and 76,437 bp, respectively, with a GC content of 48.5%. They separate encode 88 and 101 ORFs, respectively, devoid of tRNAs, virulence genes, and resistance genes. Phylogenetic analyses using VIRIDIC, Viptree, and conserved proteins suggested that these phages are closely related to *Mardecavirus*, indicating their status as new members of *Caudoviricetes* and *Mardecavirus*. Animal tests were conducted to evaluate the therapeutic effects of phage cocktail, monoclonal phage and florfenicol on *V. parahaemolyticus* (*pirA+* and *pirB+*) infections in *C. quadricarinatus*, after the establishment of *V. parahaemolyticus* infection disease model in *C. quadricarinatus*. The treatment group was able to increase the survival rate of *C. quadricarinatus*, and the phage cocktail group had a higher survival rate than the antibiotic group, with a lower survival rate of single phages. In the infected group, the hepatopancreas showed lesions such as almost disappearance of stellate structure, enlarged lumen structure, increased area of vacuoles, scattered nuclei, and interstitial blood infiltration, etc. Phages could effectively alleviate the pathological damage of the hepatopancreas, and the cocktail group and antibiotic group basically recovered from the hepatopancreatic pathological damage in 24 h, and the monoclonal phage group recovered the damage in 48 h. The treatment group had a higher survival rate than the antibiotic group in 6 h. The monoclonal phage group had a lower survival rate than the infected group. Compared with the infected group, the activities of SOD, PPO and T-AOC were significantly up-regulated at 6 h (p < 0.05), the activities of LZM were significantly up-regulated at 12 h and 24 h (p < 0.05), and the activities of PPO, T-AOC and LZM were significantly up-regulated at 48 h (p < 0.05) in the treatment groups. The expressions of ALF and Laccase-1-like protein were significantly up-regulated in the treatment groups at 6 h. The expressions of ALF and Laccase-1-like protein in the cocktail groups and the antibiotic groups were significantly up-regulated. protein expression was significantly up-regulated (p < 0.05), Lysozyme was significantly up-regulated (p < 0.05) in the cocktail and florfenicol groups, SOD was significantly up-regulated (p < 0.05) in the florfenicol group, the expression of C-type Lectin and Lysozyme was significant up-regulated (p < 0.05) in the treatment groups at 12 h, the expression of Akirin was significantly up-regulated in the treatment group (p < 0.05). It indicated that the treatment group could activate the vitality of antioxidant enzymes and lysozyme and promote the transcription and expression of related immune genes, so as to improve the immune level of the organism. The comprehensive evaluation found that the phage cocktail group and florfenicol treatment group had similar therapeutic effects, and the therapeutic effect of single phage was slightly worse.

**Conclusion:** This study demonstrated that phages have the potential to treat *V. parahaemolyticus* infections in *C. quadricarinatus*, and the phage cocktail preparation can be used as an alternative to antibiotics for further promotion and application, which provides a scientific basis for the development of a new strategy of *V. parahaemolyticus* bioprevention and control.

**Keywords:** *V. parahaemolyticus*; phage; biological properties; genomics; therapeutic effect