Formulation of a phage cocktail derived from nucleus-forming phages targeting AHPND-causing *Vibrio parahaemolyticus*

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Abstract: Acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus* (VP$_{AHPND}$) has recently been emerged and directly contributed to huge economic loss in shrimp industry. The control of the spread of this aquatic pathogen is difficult due to the emergence of multidrug resistance. Thus, alternative effective ways are urgently needed. Bacterial viruses (bacteriophage or phage) then become potential therapeutic agents for controlling the drug-resistant pathogens. With the aim to obtain a phage cocktail for controlling a wide range of VP$_{AHPND}$, we exploited a semi-high throughput screening method searching for phages and determined the potency of appropriate candidates for the cocktail. Out of 97 seawater sources screened against 28 VP$_{AHPND}$ strains, 71 positive interactions, where putative plaques were seen, were identified. We focused on only 10 interactions where the appearance plaque was clear in which it tends to be produced by lytic phages. After isolation and purification, we obtained 13 different phages that displayed different host range. Among them, phage L061A and L134 revealed the broadest host range with slightly different host specificity. Phage L061A and L134 were then investigated on both conventional and genomic studies. The result suggested that they were giant lytic phages, those with the genome size larger than 200kb, that mechanistically replicate inside the host by assembling a phage nucleus. VP$_{AHPND}$ growth inhibition revealed that the cocktail of these nucleus-forming phages had high pathogen inhibition effectiveness by suppressing the cell growth for 18 hours, suggesting the potential use as a biocontrol against various strains of VP$_{AHPND}$.

Keywords: Bacteriophage; *Vibrio parahaemolyticus*; Phage cocktail; nucleus-forming phages

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