FEASIBILITY OF BACTERIOPHAGE THERAPY FOR MITIGATING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN SHRIMP AQUACULTURE

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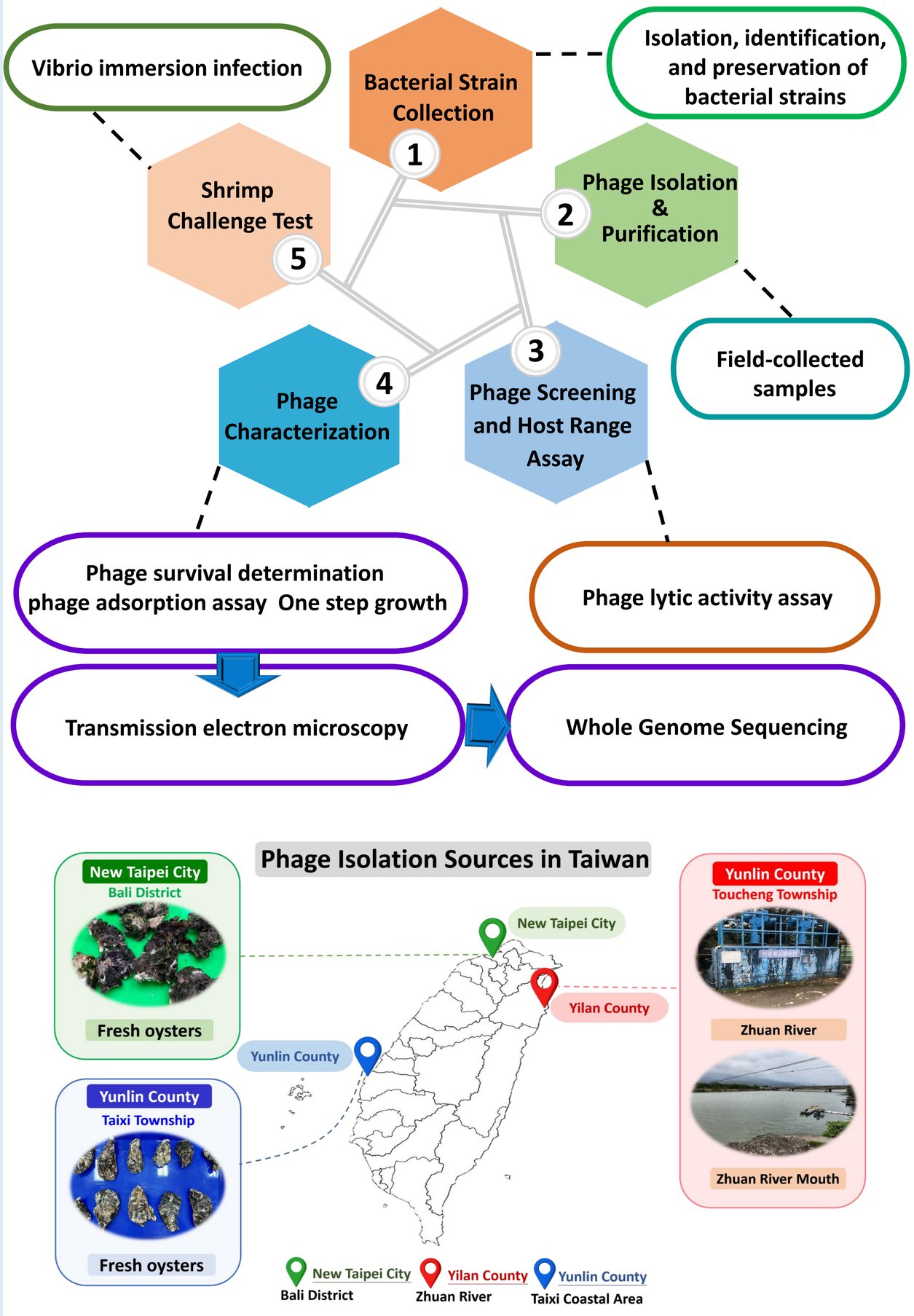
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Abstract

Acute hepatopancreatic necrosis disease (AHPND), caused by virulent strains of *Vibrio parahaemolyticus* (VP_{AHPND}), remains a significant threat to global shrimp aquaculture for which effective treatments are currently lacking. To address the risks of antibiotic resistance, this study evaluates "phage therapy" as a sustainable alternative. We isolated 23 phages targeting *V. parahaemolyticus*, identifying two candidates—BP14 and BP15—with superior lytic capacity and speed against both $Vp_{non\text{-}AHPND}$ and VP_{AHPND} isolates. Characterization through whole-genome sequencing and electron microscopy revealed that BP14 belongs to the *Inoviridae* family, while BP15 belong to the *Siphoviridae* family. Notably, BP14 demonstrated high specificity and sensitivity toward VP_{AHPND} strains.

In vivo challenges in shrimp demonstrated that treatment with these phages significantly reduced pathogenic bacterial loads and enhanced overall survival rates. These findings suggest that phage therapy provides a promising biocontrol strategy for managing bacterial infections in aquaculture and improving the safety of aquatic product processing.

Materials and methods



Result

Table 1. The lysis ability of BP1-BP23 phages towards different VP strains

Strain	$VP_{Non\text{-}AHPND}$					VP_{AHPND}				
	SO2	M2-36	BCRC 12865	BCRC 12864	BCRC 12959	3HP	5HP	China	PD2	M1-1
BP1	++	-	++	+++	+++	-	+	+++	-	-
BP2	++	-	++	+++	+++	-	-	+++	-	-
BP3	++	+	+	+++	+++	+	+	++	-	+
BP4	++	+	+	+	++	+	+	+	+	+
BP5	++	-	-	+++	+++	-	-	+++	-	-
BP6	++	+	-	+++	+++	-	+	+++	-	-
BP7	++	-	++	+++	+++	-	+	+++	-	-
BP8	++	-	++	+++	+++	-	+	+++	-	-
BP9	++	+	-	+++	+++	-	+	+++	-	-
BP10	++	+	+	+	+	+	+	+	+	+
BP11	+	+	+	+	+	+	+	+	+	+
BP12	+	+	+	+	+	+	+	+	+	+
BP13	+	+	+	+	+	+	+	+	+	+
BP14	+	++	++	++	+++	+++	+	+	+++	+
BP15	-	-	-	-	++++	-	-	-	-	-
BP16	-	-	++	-	-					
BP17	-	-	++	-	-	-	-	-	-	-
BP18	-	-	++	-	-	-	-	-	-	-
BP19	-	-	-	-	+++	-	-	-	-	-
BP20	-	-	-	-	+++	-	-	-	-	-
BP21	-	-	-	-	++	-	-	-	-	-
BP22	-	-	+++	-	-	-	-	-		-
BP23	-	-	-	-	++	-	-	-	-	-

+: plaque forming; +++: clear zone; ++: semi-turbid zone; +: turbid zone; -: plaque non-forming

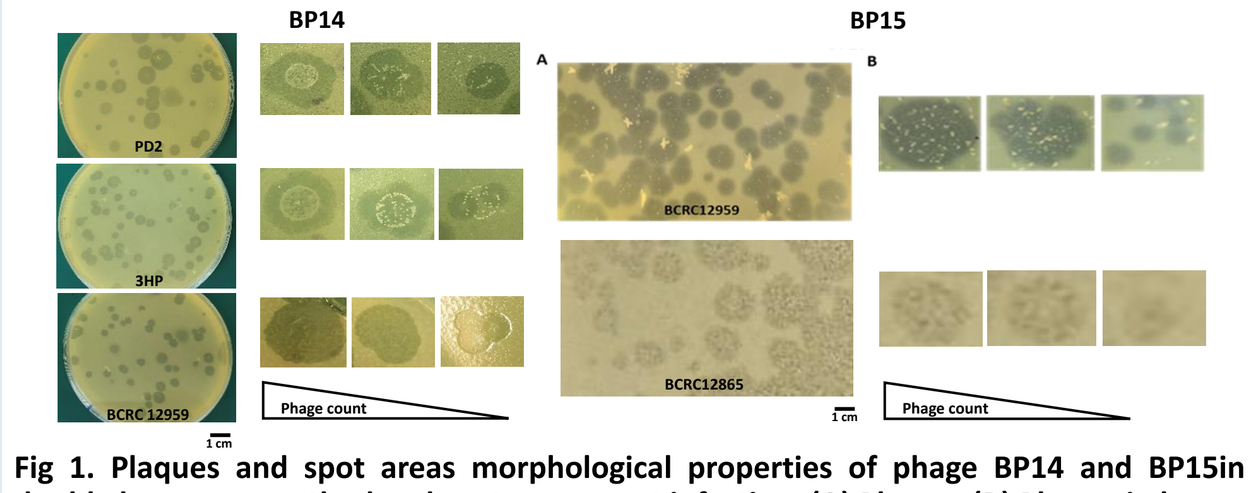


Fig 1. Plaques and spot areas morphological properties of phage BP14 and BP15in double layer agar method and spot assay post infection. (A) Plaque; (B) Phage circle.

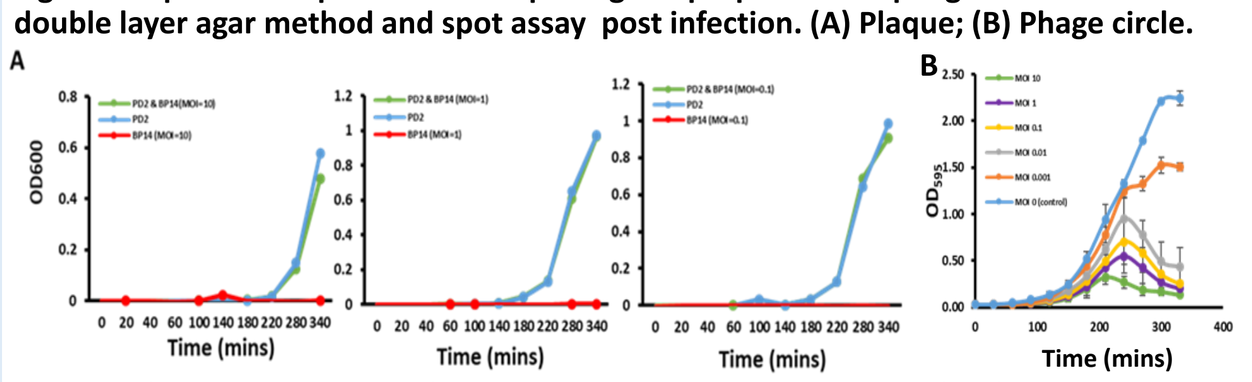


Fig 2. Infection dynamics of phage at various multiplicities of infection against *V. parahaemolyticus*. (A) BP14 infected with VP-PD2; (B) BP15 infected with VP-12959.

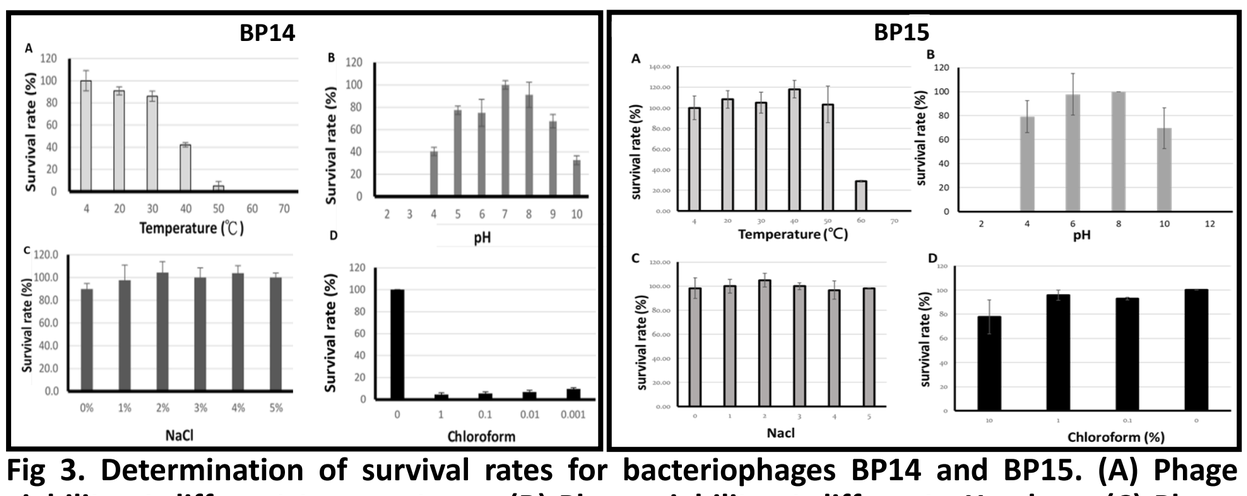


Fig 3. Determination of survival rates for bacteriophages BP14 and BP15. (A) Phage viability at different temperatures; (B) Phage viability at different pH values; (C) Phage viability at different salinities; (D) Phage viability at different chloroform concentrations.

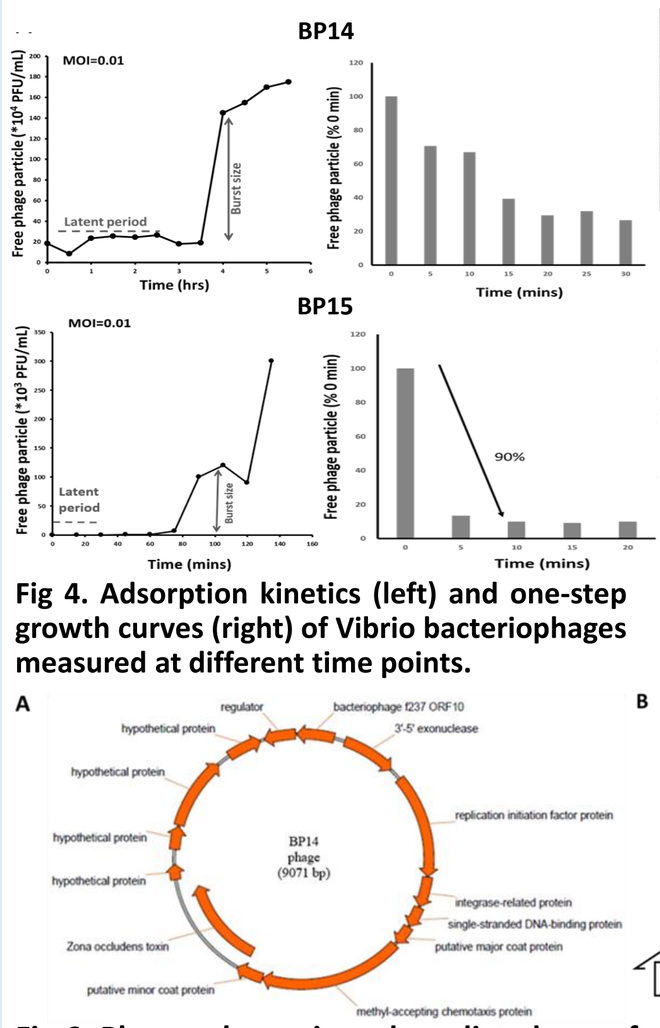


Fig 4. Adsorption kinetics (left) and one-step growth curves (right) of Vibrio bacteriophages measured at different time points.

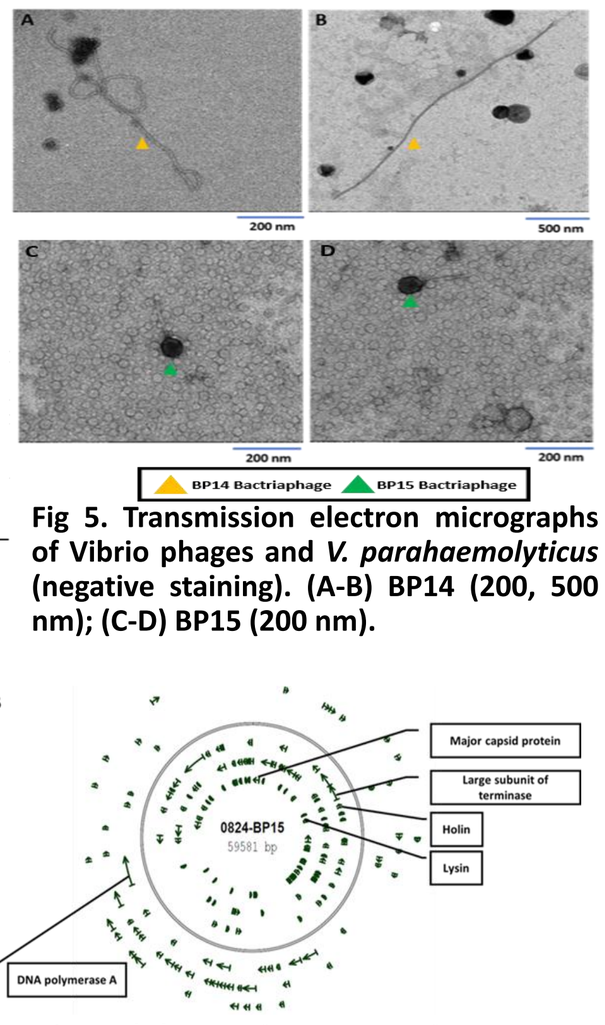


Fig 5. Transmission electron micrographs of Vibrio phages and *V. parahaemolyticus* (negative staining). (A-B) BP14 (200, 500 nm); (C-D) BP15 (200 nm).

Fig 6. Phage schematic and predicted gene functions. (A) BP14 has a 9,071 bp genome, classified as a small-genome phage. (B) BP15 has a 59,581 bp genome, classified as a large-genome phage.

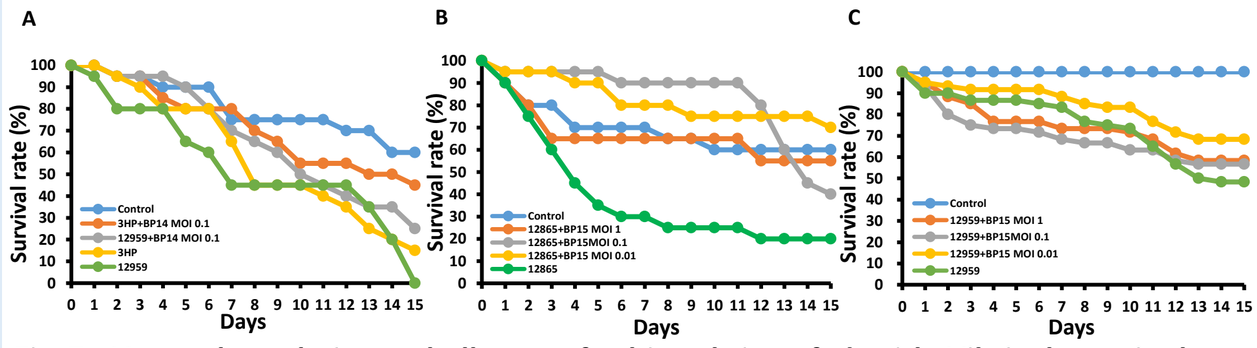


Fig 7. *V. parahamolyticus* challenge of white shrimp fed with Vibrio bacteriophage supplemented diets (n = 20). (A) Phage BP14 challenged with VP_{3HP} and $VP_{BCRC12959}$; (B) Phage BP15 challenged with $VP_{BCRC12865}$; (C) Phage BP15 challenged with $VP_{BCRC12959}$.

Discussion

Bacteriophages are natural antibacterial agents that regulate bacterial populations by inducing bacterial lysis. They exhibit high host specificity, which represents a major advantage over antibiotics. In this study, the lytic activities of bacteriophages against *Vibrio parahaemolyticus* strains VP_{AHPND} and $VP_{non\text{-}AHPND}$ were investigated. BP14 showed a broad host range but a relatively slow lytic rate and was identified as belonging to the family *Inoviridae*. In contrast, BP15 belongs to the family *Siphoviridae*, exhibiting a shorter latent period and higher lytic efficiency. These phages demonstrated strong adsorption ability and high specificity toward target Vibrio strains. However, their stability decreased under high temperatures or extreme pH conditions. Chloroform tolerance assays indicated that BP15 was highly resistant. Animal trials revealed that feeding shrimp with phage-supplemented diets significantly improved survival rates following Vibrio infection. Overall, BP14 and BP15 are promising phage candidates that effectively inhibit pathogenic Vibrio growth and may serve as alternative or complementary strategies for disease management in shrimp aquaculture. Further studies are required to assess their long-term stability, ecological safety, and potential effects on host organisms, thereby strengthening the feasibility of phage therapy and promoting sustainable, low-antibiotic aquaculture practices.

References

- Hsu et al. (2024). Isolation and characterization of the novel phage BP14 for lysing *Vibrio parahaemolyticus* and reducing virulence proteins. *Aquaculture* 581, 740484.
- Hsu et al. (2025). Characterization and genome analysis of a novel phage BP15 infecting *Vibrio parahaemolyticus*. *Scientific Reports* 15, 2801.