

Bacteriophages in shrimp hatchery: An Indian experience

Ramesh Kumar.D, Vijay Anand.D, Mathan Kumar.M, Bhaskaran.V, Salem Microbes

Sustainability in aquaculture demands a thorough and sophisticated disease management plan in which the issue of pathogenic Vibrios should be an integral part. The term Vibriosis describes both local and more general septicaemic infections caused by the members of the bacterial genus *Vibrio*. Infection caused by pathogenic or opportunistic *Vibrio* bacteria can be devastating during shrimp larval production and grow-out.

Various studies have been attempted over the past four decades to find a remedy for Vibriosis. Among them, antibiotics and probiotic bacteria have proved their efficacy in reducing the incidence of Vibriosis but with limitations. There is a need for a broad, specific and quick-acting remedy to circumvent these pathogens and prevent the disease.

Why bacteriophages?

Bacteriophages are viruses that are natural predators of bacteria, self-limiting and self-replicating in their host cell, and can evolve with resistant bacteria to combat them. They are commonly found in large numbers wherever their hosts live, in natural bodies of water and aquaculture operational areas. The use of phages as biological control of pathogens of cultured shrimp has developed an interest in recent years since no drug residues or drug toxicity is associated with them.

Articulated from the probiotic research and shrimp disease diagnostic experience, Salem Microbe's Research and Development team has developed a bacteriophage-based product, V Phages Hatchery, for shrimp hatcheries. This phage formulation aims to reduce pathogenic *Vibrio* species load in the rearing environment without affecting the beneficial microflora, leading to an improved survival and growth rate of PLs.

The product can be broadly described as a cocktail of lytic bacteriophages against pathogenic *Vibrio* species

present in the aquaculture environment, such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio campbellii* and other pathogenic *Vibrio* spp. The members of this cocktail belong to the viral families *Siphoviridae*, *Myoviridae* and *Podoviridae*.

A variety of delivery routes has been attempted and finally, the product has been designed as a liquid formulation for ease of application with minimum loss of activity retaining biological efficacy.

The V Phages Hatchery formulation was tested in different stages of commercial *L. vannamei* shrimp hatcheries in India to verify its efficacy, and some of the results are presented in this article.

Application in broodstock tank

Fecal strand samples from broodstock tanks were taken for microbiological analysis that confirmed the presence of pathogens *V. harveyi*, *V. campbellii*, *V. parahaemolyticus*, *V. alginolyticus* and *V. neocaledonicus*. Broodstock tanks were then treated with bacteriophages to reduce the *Vibrio* load.

To check the effectiveness of bacteriophages, these tanks were dosed as per the standard recommendation of commercial bacteriophage product. Fecal strands were sampled 24 and 48 hours after the treatment, and analysis showed a reduction in *Vibrio* load by 2 to 4-log, in certain cases up to 5-log reduction (Fig. 1,2). Simultaneously, an increase in gram-positive bacteria was also observed. This increase in the number of potential probiotic strains that have been routinely applied in these tanks is a positive effect of the suppression of the *Vibrio* population leading to increased substrate availability for the probiotics to thrive.

Application in Artemia tank

The plate count analysis of two *Artemia* water tanks

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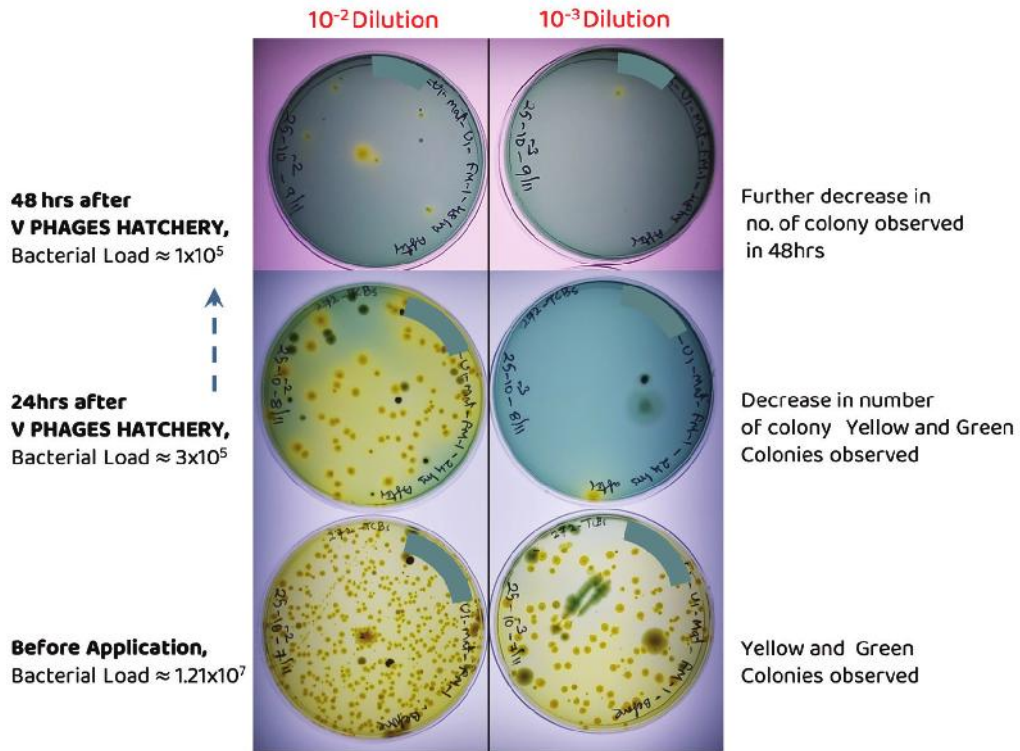


Figure 1. TCBS plating for Vibrio load enumeration of fecal strands in broodstock tanks

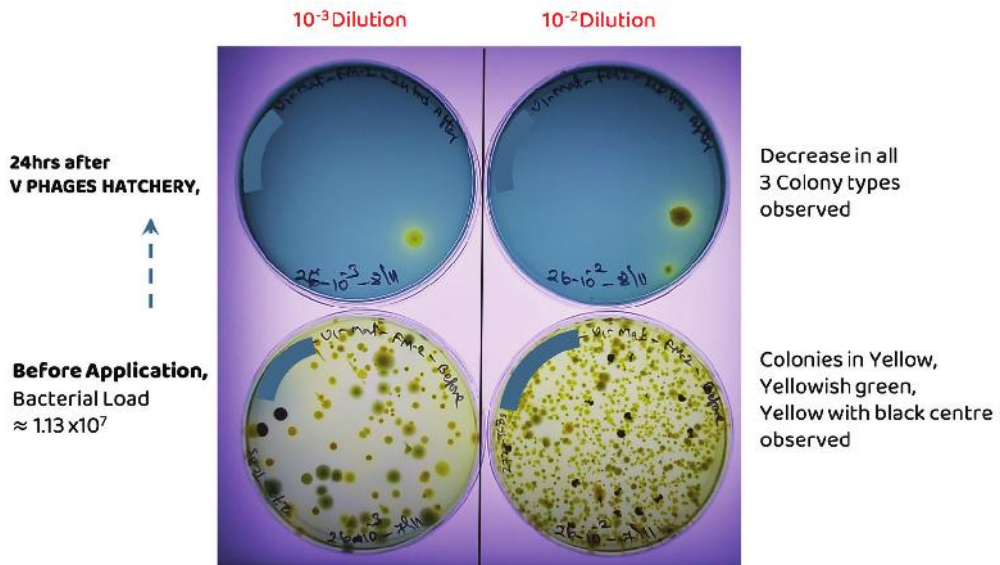


Figure 2. TCBS plating for Vibrio load enumeration of fecal strands in broodstock tanks

before application of V Phages Hatchery showed 4 and 5 morphotypes comprising gram-negative rods with bacterial load ranging from 9.3×10^7 to 2.4×10^8 CFU/mL in TSA and 2.5×10^7 CFU/mL in TCBS of the two samples (Table 1).

Eighteen hours post application of V Phages Hatchery, three different morphotypes were observed in two samples plated on TSA with microbial load ranging from 9.8×10^7 to 1.09×10^8 CFU/mL. The TSA plates were dominated by gram-positive bacteria. A reduction of

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Table 1. Comparison of microbial load of two Artemia tanks before and after application

Condition	Tank No.	TSA (CFU/mL)	TCBS (CFU/mL)	Morphotypes in TCBS
Before application	1	2.4×10^8 (5 types)	2.5×10^7 (4 types)	4 types (G-ve rods)
	2	9.3×10^7 (4 types)	2.8×10^7 (4 types)	4 types (G-ve rods)
After application	1	9.8×10^7 (3 types)	2.9×10^7 (1 type)	1 type (G-ve rods)
	2	1.0×10^8 (3 types)	1.15×10^7 (1 type)	1 type (G-ve rods)

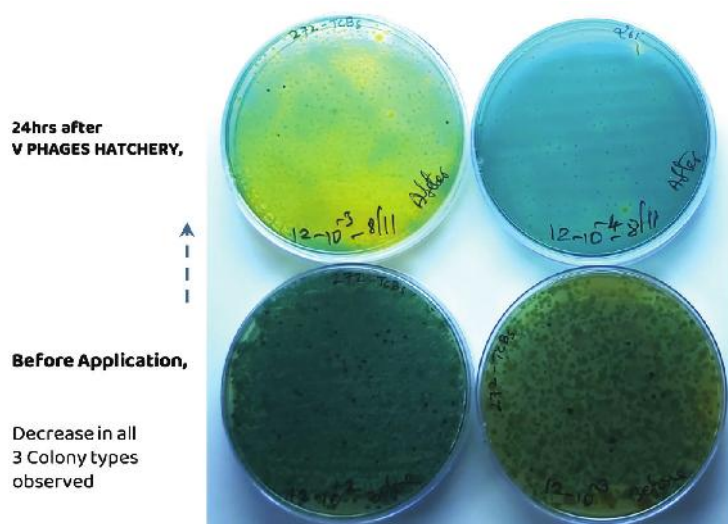


Figure 3. TCBS plate showing morphotype reduction of Artemia tank samples in TCBS plates

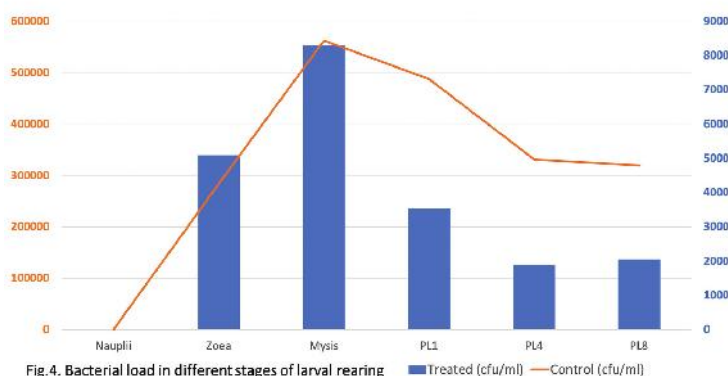


Figure 4. Water bacterial load of the larval tanks treated and untreated with bacteriophage

morphotypes from 4 to 1 type of gram-negative strains in TCBS plates was also observed in both the samples with load ranging from 1.15 to 2.9×10^7 CFU/mL with the dominant group being a single morphotype comprising gram-negative rods (Table 1).

The absence of known pathogenic *V. alginolyticus*, *V. parahaemolyticus* and *V. campbellii* in the treated tanks showed the broad spectrum lytic activity by V Phages

Hatchery application. The load reduction can be termed as “smart disinfection” (Fig. 3).

Application in larval rearing tanks (LRT)

A total of 24 tanks were treated in two different hatchery systems with duplicates and control to measure the impact of bacteriophages as a prophylactic aid. The product was administered as per the standard recommended dose of the commercial product during Nauplii stocking. Water and animal samples were drawn at each conversion stage and analyzed for bacterial load until the PL packing stage.

No bacterial load was detected in the Nauplii stage tank water before stocking in both the control and treated systems. Samples of Zoea stage tank showed a water microbial load of 2.8×10^5 CFU/mL in the control tank, while the treated tank water showed 2-log reduction in microbial load of 5.1×10^3 CFU/mL. A similar pattern was observed in Mysis, PL1, PL4, and PL8 with an average of 2-log reduction in microbial load of bacteriophage-treated tank water (Fig. 4).

While analyzing Nauplii stage crushed animal samples immediately after stocking and before the application of bacteriophages, it showed a bacterial load of

1×10^6 CFU/mL. Twenty-four hours post-application, samples showed no reduction in CFU/mL but the bacterial flora composition changed from green-dominating colonies to yellow-dominating colonies of gram-positive species (Fig. 5).

In another tank study, the microbial load of the animals was tested at various stages from Nauplii to PL8. The whole Nauplii crushed sample showed 1.3×10^2 CFU/

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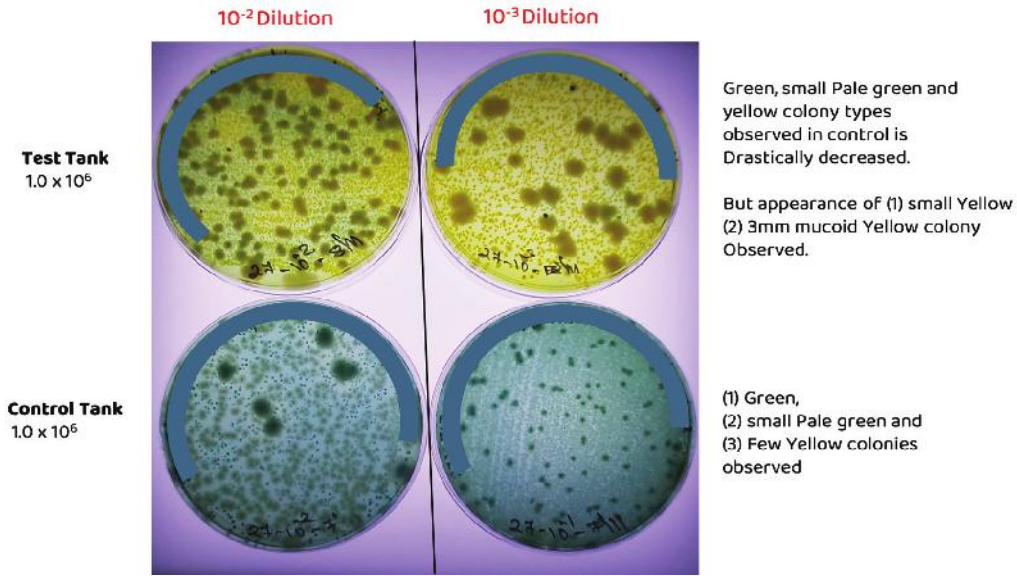


Figure 5. Bacterial load of the Nauplii tanks treated and untreated with bacteriophage

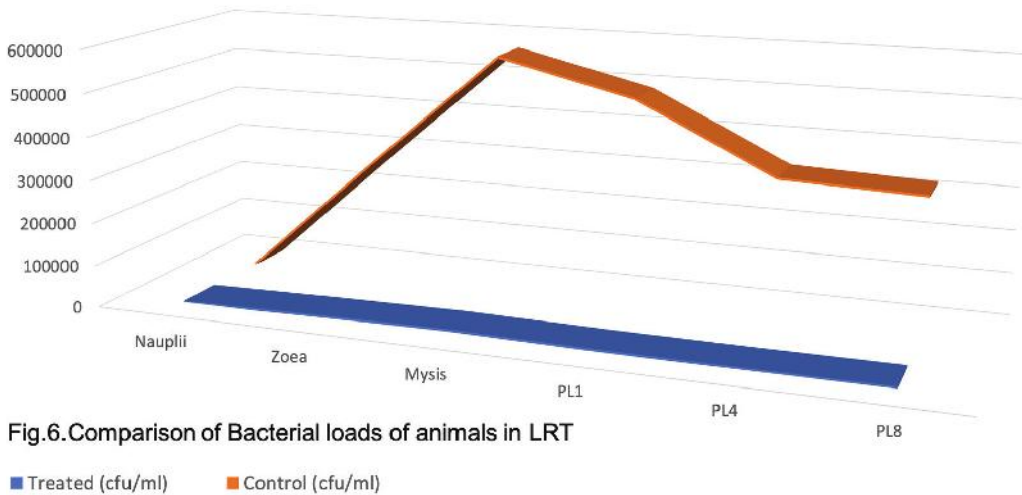


Fig.6. Comparison of Bacterial loads of animals in LRT

Figure 6. Animal bacterial load of the larval tanks treated and untreated with bacteriophage

mL of microbial load in TCBS plates in both treated and control tanks before bacteriophage application. As the stage progressed, there was an increase in bacterial load in the control tank and a reduction of 2 to 3-log was observed in treated animal samples. The control tanks had 10^5 - 10^7 CFU/mL, while the treated tanks had significantly less microbial load, 10^3 - 10^5 CFU/mL (Fig. 6).

When the crushed animal microbial load was analyzed at PL4 stage, the control tank showed 6.1×10^5 CFU/

mL with a mixture of yellow and green colonies. While the treated tanks had 1.3×10^5 CFU/mL with a considerable reduction in green colonies (Fig. 7).

The survival rate of animals in larval rearing tanks was higher than in the control tank. No significant differences in survival rate were found in Nauplii and Zoea stages, but as the stages progressed, a nearly 20% increase in larval survival rate was observed at Mysis, PL1, PL4, and PL 8 stages (Fig. 8).

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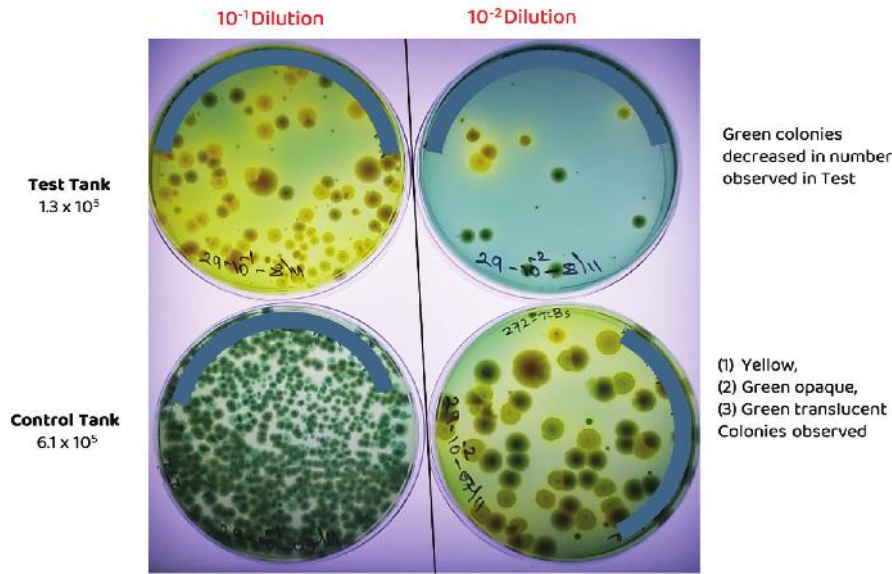


Figure 7. Animal bacterial load of the PL4 tanks treated and untreated with bacteriophage

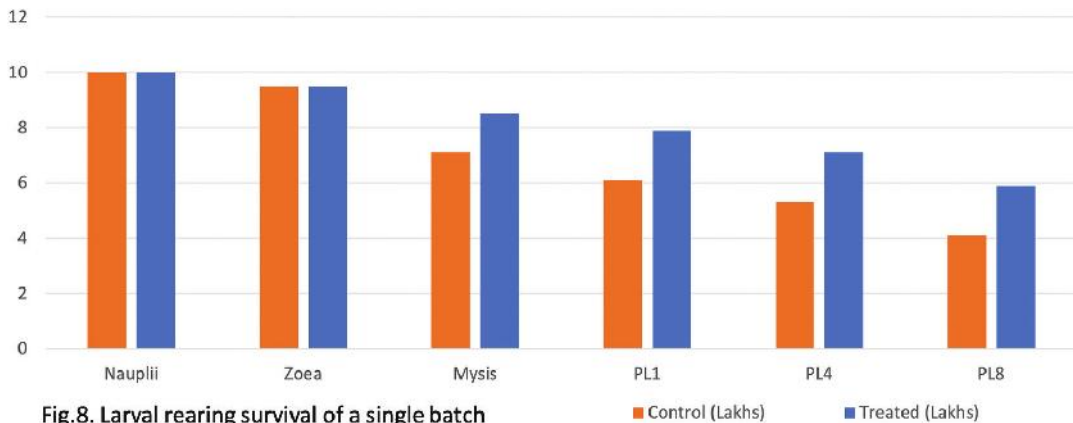


Fig.8. Larval rearing survival of a single batch

	Nauplii	Zoea	Mysis	PL1	PL4	PL8
Control (in Lakhs)	10	9.5	7.1	6.1	5.3	4.1
Treated (in Lakhs)	10	9.5	8.5	7.9	7.1	5.9

Figure 8. Comparison of the survivals during various stages of LRT

Conclusions

Phages lead to a reduction in the *Vibrio* load and in slime and biofilm formation in larval tanks, reducing the reservoirs of pathogens. The phage therapy proves to be a promising additional amendment in controlling *Vibrios* in aquaculture, having the important advantage of not affecting the beneficial microbiome of the shrimp and its rearing system.

References available on request

More information:

Dr. RAMESH KUMAR DHANAKOTI
 Chief Executive Officer/Head R&D
 Salem Microbes Pvt. Ltd.
 E: ramesh@salemicrobes.com

