

Bacteriophage therapy in shrimp farming

Case studies on the effectiveness of bacteriophages during vibriosis in shrimp grow out in India

By D. Ramesh Kumar, M. Mathan Kumar, Sasibhusan Manna, Chandan Kumar and Sudharsan Das

Recent scientific research has brought to light the importance of maintaining good and stable microbiome in shrimp and fish throughout the culture cycle. Amidst current disease challenges, we believe that bacteriophages will be helpful against virulent pathogens. They can maintain the natural ecosystem of the pond while mitigating effects of pathogens and without damaging the essential microbiome population.

Salem Microbes Pte Ltd started research on bacteriophage therapy in 2018. Supported by a strong R&D team, qualified field technical staff and an aquaculture laboratory, the bacteriophage consortia for shrimp hatchery and shrimp grow out were launched in early 2023. Today, we are strong in bacteriophage research, specialising in isolation, characterisation and formulation of phage cocktails within a short turnaround. Our R&D team ensures quick screening during sudden disease outbreaks and develop bespoke formulation of bacteriophages from our expansive library of bacteriophages maintained on-campus.

Phages and probiotics, a synergistic combination

Probiotics reduce the chances of infection by competitive exclusion, but once the critical levels of *Vibrio* species dominate the environment, it becomes nearly impossible for probiotic bacteria to exclude the pathogenic bacteria. A quick intervention is required during such critical conditions. This should be non-residual and non-GMO in nature, and which does not affect the microbiome. This is the concept of phage therapy which is highly specific and quick acting specifically targeting aquaculture pathogens.

When is a bacteriophage product relevant?

A good bacteriophage consortia should have identified lytic phages, specific against pathogens selected from the target environment. Phage cocktails against pathogens should be continuously upgraded to maintain the pathogen spectrum and uphold specificity and infectivity (Figure 1).

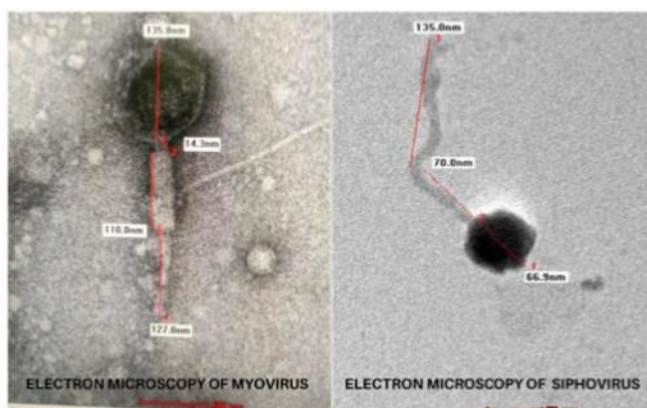


Figure 1. Electron microscopy of V phages consortia members.

The cocktails of V phages for hatchery and grow out are quite different in their formulation, specificity and purpose. The product V Phages Hatchery targets the specific pathogens prevalent in the hatchery and water source, live feeds and its environment. V Phages Growout targets the common pathogens especially *Vibrio* spp. which are the main causes for bringing down the productivity of a shrimp pond by causing vibriosis. Shrimp show signs of poor appetite, slow growth, loose shell, damage in the hepatopancreas, white gut disease, white faeces disease (WFD), and running mortality syndrome (RMs), all of which lead to crop losses.

V Phages cocktail targets *Vibrio* species such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio campbellii* and other pathogenic *Vibrio* spp. (Figure 2)

The product V Phages Growout was launched in early 2023, after extensive trials under controlled conditions. It was distributed for public use with a technical awareness program and farmer meetings. We have recommended its use as a prophylactic treatment to avoid current challenges with RMS, white gut disease and WFD which are caused specifically by severe infections of *Vibrio* spp. However, the use of the product is at the discretion of each farmer based on the stage of culture and risk of crop loss. Below we describe some case studies.

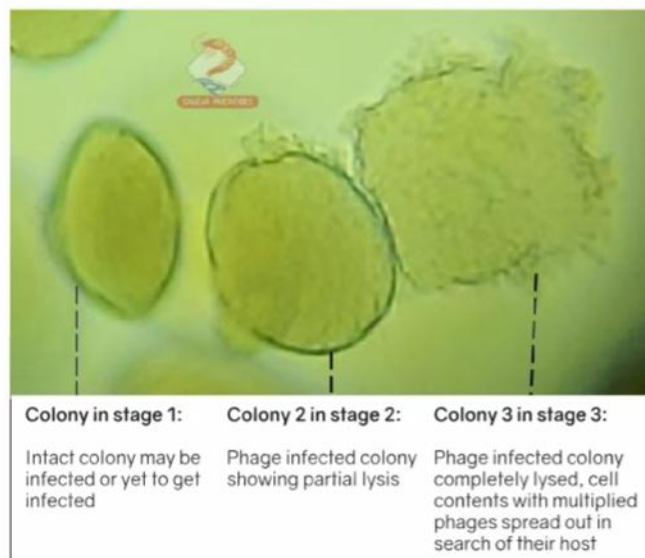


Figure 2. Stages of phage infected *Vibrio* colonies.

Case study 1: White gut disease

In the Pramanik aquafarm in Nankar village, Chandaneswar, Odisha, owner Umakanta Behera, is a farmer with 16 years of experience. The farm was selected to demonstrate the product. Umakanta also owns a retail outlet to supply feeds and health products to other farmers.

The farm has 12 ponds with sizes ranging from 1,500m² to 3,000m² water surface area (WSA). *Litopenaeus vannamei* post larvae (PL10) were stocked in three nursery ponds. These were later transferred to nine ponds after 34 days (DOC 34) on attaining average weights of more than 1g.

During culture, four ponds were infected with vibriosis (pond Nos. 1,2,11,12) which were adjacent to one another sharing bunds, and showed symptoms of white gut disease between DOC66 and DOC69. This infection correlated with a drop in feed intake of 6 to 14kg/day over 7 days. Shrimp tested negative for *Enterocytozoon hepatopenaei* (EHP) and V Phages Growout application was recommended as a therapeutic treatment.

In this case, the primary aim of bacteriophage application was to reduce the spread of the infection to the other shrimp in the same pond and/or nearby ponds. It was also to prevent mass mortality, thereby extending the culture cycle.

The application of the bacteriophage resulted in the following (Figure 3).

- The number of shrimp showing white gut disease was reduced to a large extent.
- There was a consistent increase in feed consumption for the next 14 days, until harvest.
- An overall drop in feed intake for 3 days was observed in all the ponds (both infected and not infected) due to the very high temperature at above 40°C.
- There was good growth performance. The farmer could harvest shrimp at DOC91 and DOC 92 at size 66/kg to size 55/kg. This was much earlier than targeted because of a price crash in May 2023.



Figure 3. Comparison on feed intake (kg), harvest (kg), density (PL/m²), average body weight (ABW in g) and survival (%) in infected/treated ponds (1,2,11,12) and uninfected/untreated ponds (4,5,8) at Pramanik aquafarm.

From the above, we concluded the following.

- The unplanned harvest which was characteristic of white gut disease was avoided.
- Analysis of harvest data of the four infected and treated ponds revealed that the loss in survival was from 26% to 20% respectively. This is assumed to have happened within the period of DOC67 (when initial feed drop was noticed) to DOC91 i.e., the harvest date.
- The spread of infection to the adjacent ponds was prevented, as there were no new outbreaks of white gut disease, during this observation period from DOC73 to DOC91.
- The cumulative feed intake and FCR of the infected ponds did not differ much from the three uninfected ponds, selected for study. This clearly states that the mortality was controlled immediately.
- V Phages Growout application has helped the farmer to extend the culture cycle during the critical breakeven and profitable period.

Treatment regime

The dosage for V Phages Growout was at 100mL/4,000m². This bacteriophage was also given as a feed supplement, concurrently in two feeds (first and last) for 7 days as per standard protocol for this product. During this cycle a heterotrophic bacterial probiotic was applied as per their regular schedule. No sanitiser or disinfectant was applied to these treated ponds.



Shrimp (16g) with white gut disease at Pramanik aquafarm in Nankar village, Chandaneswar, Odisha.

Case study 2: Running mortality syndrome

Venu owns a 3-acre (1.21ha) pond in Nidamarru village, West Godavari District, Andhra Pradesh. He stocked 7 lakhs (700,000) of *L. vannamei* post larvae. RMS occurred from DOC35, and mortality averaged 60 shrimp/day. The diseased shrimp were tested negative for EHP with PCR but were positive with high levels of vibriosis infection. V Phages Growout was applied at 100mL /4,000m² in water at DOC41 followed by adding the product as a feed supplement in 4 feeds/day from DOC42 onwards. There was a positive sign of recovery with mortality reducing to seven shrimp from day 3 after the treatment.

BACTERIOPHAGE THERAPY TO CONTROL PATHOGENIC VIBRIOS

V PHAGES HATCHERY & V PHAGES GROW-OUT are cocktails of phages isolated from the natural environment. They destroy the pathogenic bacteria, including those resistant to antibiotics and increase the efficacy of probiotics.

V PHAGES HATCHERY

EFFECTIVE ON SUPERBUGS

- Assures a *Vibrio-free* environment throughout the hatchery cycle.
- Establishes favourable conditions to produce High health Post Larvae.



Targeted *Vibrio* sp.

- *Vibrio parahaemolyticus* • *Vibrio alginolyticus* • *Vibrio harveyi*
- *Vibrio campbellii* and other pathogenic *Vibrio* sp.



V PHAGES GROW-OUT

EFFECTIVE ON SUPERBUGS

- Effective against Vibriosis, White Faeces and Running Mortality Syndrome.
- *Vibrio-free* conditions improve probiotic performance and shrimp growth.



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There was a drop in mortality and absence of RMS in shrimp with the application of the bacteriophage. There was a large improvement in feed intake. On DOC 46, a partial harvest of 2 tonnes was achieved. It was observed that shrimp showing signs of RMS infection was considerably reduced at harvest, indicating the containment of infection due to targeted bacteriophage treatment against vibriosis.

Case study 3: White faeces disease

Bijaykumar Nayak owns a 5ha farm with 20 ponds of varying sizes in Silda village, Balasore district, Odisha. This farm was stocked with *L. vannamei* post larvae in February 2023. Two months after stocking, the farm gradually experienced WFD. We used pond No 2 as a case study. In this 5,000m² pond we observed very few floating white faecal strands at DOC72 which increased drastically at DOC78. The symptomatic animals when tested were EHP negative. Vibriosis was suspected and V Phages Growout was applied at 100mL/4,000m².

Subsequently, by DOC85, the number of floating white faecal strands was reduced drastically but was still appearing in check trays. We suggested to the farmer to apply a second dose at DOC86 at the same dosage. By DOC90 white faecal strands almost stopped appearing. The pond was harvested at DOC103. Until this period the daily feed rate was consistently increasing with no drop in the daily feed ration. The average weight of the shrimp was 16.5g at harvest. The estimated survival rate was 92%.

We observed that this is a strange case where even with large numbers of white faecal strands floating, hardly any shrimp mortality was observed. The average body weight was always increasing and reached an average of 0.2g/day after the first dose and was 0.23g/day after the second dose of V Phages Growout.

Overall, the weight increases were from 10.53g to 16.50g between DOC78 and DOC103 (i.e., 25 days) which translated to a biomass increase of 1.65 tonnes. The total harvest was 4.57 tonnes which could not have happened without this therapeutic phage intervention. It also prevented mass mortality and avoided a forced harvest.

References are available on request



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"V PHAGES GROWOUT" cocktail targets against most common pathogenic *Vibrio species* in Shrimp Farming

- Vibrio parahaemolyticus*
- Vibrio alginolyticus*
- Vibrio harveyi*
- Vibrio campbellii* and other pathogenic *Vibrio sp.*



Lysis of pathogen and release of new phages for repetitive action

VIBRIOSIS



Use immediately when various symptoms of Vibriosis as Luminescence in water, infections in Shrimp and or Low feed intake etc. correlated with by lab test.

WHITE FAECAL DISEASE



Use immediately on observation of few strands on White fecal matter floating on water, or on check tray. Start treatment.

WHITE GUT DISEASE



Use immediately on observation of few shrimps with White Gut, in check tray or sampling net. If more than 5 days of White Gut Disease is reported, there is high chance of spread in soil and water and so treatment takes time to respond.

RUNNING MORTALITY SYNDROME



Use immediately on observation of dead pieces which is normally more than the regular Natural dead pieces observed in that pond on a daily basis. This will first control RMS due to VIBRIOSIS within 3 days and then reduce.



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V PHAGES HATCHERY is a cocktail of Phages isolated from Natural environment. Hence they are safe on aquatic animals, people and ecosystems. This destroys pathogenic bacteria which are even resistant to antibiotics and increases the efficacy of probiotics.

"V PHAGES HATCHERY" cocktail targets against most common pathogenic *Vibrio* species in Shrimp Hatchery.

- Vibrio parahaemolyticus*
- Vibrio alginolyticus*
- Vibrio harveyi*
- Vibrio campbellii* and other pathogenic *Vibrio* sp.

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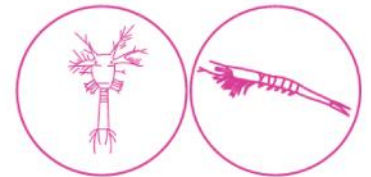
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Prevents entry of opportunistic pathogens and safeguards health of this high value asset.



Artemia:

Reduces the *Vibrio* sp. load in Artemia tank.



Zoea & Mysis:

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Stagewise control of *Vibrio* sp. results in remarkable reduction of *Vibrio* sp. load in post larval tanks. This results in high health seeds.

One Health unified approach for balanced welfare of people, animals and ecosystems.

Works as an Alternative to Antibiotics and complies with International Seafood export regulations.



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