Isolation and Characterization of *Vibrio harveyi*-infecting Bacteriophages

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Submitted by

Papiya Munshi, Ahan Samanta, Avijit Das, Sagar Jana, Anjusree Maji, Sisir Dhauria

Under supervision of

Dr. Kuntal Ghosh
Assistant Professor in Microbiology and TIC
Department of Biological Sciences

MIDNAPORE CITY COLLEGE
Kuturiya, P.O. Bhadutala,
Paschim Medinipur, Pin-721129
West Bengal, India

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CERTIFICATE

This is to certify that the project report entitled ***'Isolation and Characterization of Vibrio harveyi-infecting Bacteriophages'*** *submitted by* **Papiya Munshi, Ahan Samanta, Avijit Das, Sagar Jana, Anjusree Maji, Sisir Dhauria, Roll – PG/VUWGP29/BOT-IVS No- 037, Roll – PG/VUWGP29/BOT-IVS No- 002, Roll – PG/VUWGP29/BOT-IVS No- 012, Roll – PG/VUWGP29/BOT-IVS No- 047, Roll – PG/VUWGP29/BOT-IVS No- 006, Roll – PG/VUWGP29/BOT-IVS No- 056** to the Midnapore City College, Midnapore, West Bengal, India during the year of 2023 *in partial fulfillment for the award of the degree of Master in Botany is a bona fide record of project work carried out by him/her under my/our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.*

Dr. Kuntal Ghosh
Assistant Professor in Microbiology
MIDNAPORE CITY COLLEGE

Teacher-in-Charge
MIDNAPORE CITY COLLEGE

Director
MIDNAPORE CITY COLLEGE

Date:

Place: Midnapore City College, Paschim Medinipur

Declaration

I do hereby declare that the present Master thesis entitled '**Isolation and Characterization of *Vibrio harveyi*-infecting bacteriophages**' embodies the original research work carried out by me in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Kuntal Ghosh, Assistant Professor, Dept. of Biological Sciences, **MIDNAPORE CITY COLLEGE,** Kuturiya, Bhadutala, Pin-721129, Paschim Medinipur, West Bengal. No part thereof has been submitted for any degree or diploma in any University. I shall not publish this work without consent of the supervisor.

Date:

Place: Midnapore City College, Paschim Medinipur

Approval Sheet

This project report entitled by “Isolation and Partial Characterization of *Vibrio harveyi*-infecting bacteriophages” by Papiya Munshi, Ahan Samanta, Avijit Das, Sagar Jana, Anjusree Maji, Sisir Dhauria,is approved for the degree of Master in Botany.

(Signature of Examiners)

(Signature of Guide)
(Dr. Kuntal Ghosh)

(Dr. Pradip Ghosh)
Director
Midnapore City College

Date:

Place:

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Abstract

India is the largest shrimp culturing country. Vast numbers of people are economically dependent on the shrimp culture. The majorly produced shrimp is the tiger shrimp (*Penaeus monodon*). *Vibrio harveyi* is the main causative agents of luminous vibriosis disease in shrimp and causes massive death of tiger shrimp and eventually responsible for economic losses. The antibiotics are majorly used to treat this infection. However, some of the countries banned to import the antibiotic-treated shrimp because of the development of antibiotic resistant bacteria. This incidence impelled the scientist to find out the alternatives of antibiotics, such as probiotic, bacteriophage, etc. Considering this, the current project aims to find out the broad host infective lytic bacteriophages to treat *V. harveyi* infection in shrimp culture.

In this project, *V.* harveyi-infecting specific broad host range lytic bacteriophage will be isolated from the water bodies of shrimp culture or the environmental sample. The phage will be propagated on the selective host under *ex-situ* condition and characterized partially. The isolation and successful application of the bacteriophage may reduce the shrimp mortality and eventually increase the earning of the shrimp cultivars.

Keywords: *Vibrio harveyi*; bacteriophage; host range; lysis; biocontrol.

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Chapter 1: Introduction

1. Introduction

Aquaculture is a promising and fastest developing food-producing sectors in India with the largest potential to accomplish growing demand for aquatic food (Das et., 2017). Indian aquaculture has immensely grown and reached the second aquaculture product producing country after China with a production of 46 lakhs tonnes in 2011 (FAO 2013). Among the aquaculture products, shrimp is one of the dominant and produce about 6 lakh tonnes of shrimps in 2018-2019 in India (Krishnakuma, 2018).

Disease outbreaks, a common phenomenon in the shrimp industry, cause a massive drop in shrimp production. The gross national losses in India due to shrimp diseases were estimated at 48,717 metric ton of shrimp valued at Rs. 1022.1 crores in 2008 (Kalaimani et al., 2013). It was reported that most of the mass mortalities of tiger shrimp are due to the bacterial infection mainly caused by *Vibrio harveyi* (Karunasagar et al., 2007). The bacterium is known to be one of the causative agents of luminous vibriosis disease in a shrimp farm. This has resulted in 50–100% mortality rate among shrimps and cause of *Vibrio* infection (Letchumanan et al., 2016). The uses of antibiotics can reduce *V. harveyi* infection and increase shrimp production. However, the presence of antibiotic residue in shrimp leads to the rejection of exporting shrimp in different countries. In 2017-2018, the US FDA refused to allow 26 types of shrimp products from India into the US due to the detection of antibiotics (Venkatasubramanian, 2019). Moreover, India's share in EU rejections stood at 62, 71, and 83% in the years 2015, 2016, and 2017 respectively (Kumar, 2018) . India's Marine Products Export Development Authority (MPEDA) has recently banned the use of antibiotics in shrimp production. The bacteriophage therapy is an effective alternative to antibiotic therapy. Some studies in the worldwide showed that the bacteriophage can be used to control *V. harveyi* infection in shrimp(Ibrahim et al., 2017; Karunasagar et al., 2007; Oakey & Owens, 2000; Vinod et al., 2006). Surprisingly, the application of bacteriophage

at the field trial stage is till now lacking in our country to prevent this detrimental bacterium.

For the successful application of the bacteriophages in the shrimp culture, the whole genome sequence analysis is very essential. This is because, the bacteriophage genomes sometimes contain the virulence-related genes or antibiotic resistant genes, which might be transferred to the pathogenic bacteria during the lysogenic growth of the bacteriophage. Therefore, it is essential to screen such a bacteriophage which does not contain any virulence-related genes or antibiotic resistant genes. Considering this, the project aims to screen an effective *V. harveyi*-infecting lytic bacteriophage and its application in shrimp culturing pond to control *V. harveyi* infection.

Bacteriophage

The initiated extensive studies of the highly symmetrical head, tails and baseplate using primarily E.M. (Kaper et al., 2004). The phages are different size and shape. The most observed group suggested that the phages have ds-DNA genome. The tailed phages have three major components: the genome packed capsid, a tail that served as a pipe during infection to source transfer of genome into host cell and a major tacky system at the of the tail that will recognize the host cell and penetrate its wall for the reproduction (Helen et al., 2019).

Life cycle of bacteriophage

On the earth, the bacteriophages are the most richly found. The bacterial viruses have genetic material in the form of DNA or RNA, which is coated by protein (Clark and March, 2006). This protein coat is known as capsid. The capsid is connected with a tail which has fibers. This tail used for attachment to receptors on bacterial cell surface. Except filamentous phages almost all the phage has polyhedral capsid (Ackerman, 1998). Phages infect bacteria and can propagate in two possible way, lytic cycle and lysogenic life cycle. When phages multiply vegetatively they kill their host and the life cycle is referred to lytic cycle (Inal, 2003). If supposition to some harsh condition like ultraviolet ray occurs then the prophage will escape via lysis of bacteria (Summers, 1999). The bacteriophage infects and complete their life cycle inside *E. coli* in the following steps:

Attachment

The bacteriophage attaches itself on the surface of bacteria. The process will know as

absorption. The tip of the ten fibers attach to the specific receptor on the surface of the bacterial cell.

Penetration

The base plate and the tail fibers are attached firmly to the bacterial cell. The phage muramidase (lysozyme) weakens a part of the cell wall and the hollow core is the pushed downwards through it. The DNA is injected inside the bacterial cell.

Synthesis

The component of new virus particles are produced after the nucleic acid is released into the cell. The subunits of phage head, tail and late protein then appeared. The synthesized is carried out by specific enzyme called early protein. The nucleus and cytoplasm also contain the component of phage.

Maturation and assembly

On maturation, the head and tail protein of phage DNA assemble and each component of phage DNA is surrounded by a protein coat. Ultimately, the tail structure are added forming a virion.

Release or lysis

The infected bacterial cell is lysed releasing the progeny phages. The phage enzymes weaken the cell wall of bacteria during replication. In this phase mature viruses burst out of the host cell in a process called lysis and the progeny viruses are liberated into the environment to infect new cells.

Bacteriophages are among the most common and diverse entities in the biosphere. Bacteriophages are ubiquitous viruses, found wherever bacteria exit. They can be found in soil and seawater, oceanic and terrestrial surfaces and extreme environments, such as those characterized by very high or very low temperatures. Moreover, they have been detected in hospitals, in wastewater and where bacteria can live, including animal and human tissues (Clokie et al., 2011).

Chapter 2: Literature Review

2. Literature Review

Bacterial diseases are a major problem affecting shrimp farming and most of the mass mortalities of shrimp are associated with luminous disease caused by *V. harveyi* in India (Karunasagar et al., 2007). *V. harveyi* is a Gram-negative bioluminescent bacterium with curved rod shape. It is motile via a single polar flagellum. Antibiotics such as Oxytetracycline, Florfenicol, Sarafloxacin, Enrofloxacin, Chloramphenicol, Ciprofloxacin, and Furazolidone are widely used in shrimp aquaculture to control bacterial infections in India. Most of the antibiotic use is not supervised by any scientific personnel and is generally based on the experience of fellow farmers. This may enhance shrimp productivity, but at the same time, the abuse of antibiotics leads to the development of antibiotic resistance bacteria. Moreover, the presence of antibiotic leads to the rejection of exporting shrimp in different countries. Considering the emergence of antibiotic-resistant pathogens and awareness of adverse effects of antibiotics has led to a need for alternatives to antibiotics in shrimp aquaculture. Bacteriophages have been suggested to be an substitute to antibiotics for the control of bacterial diseases in aquaculture (Almeida et al., 2009).

INTERNATIONAL STATUS:

Most of the work on the *V. harveyi*-infecting bacteriophages have conducted in Asian countries such as Malaysia, Thailand, etc. The details of the major findings are tabulated in Table 1.

Table 1. Potential application of bacteriophage for controlling *Vibrio harveyi* worldwide.

Aquaculture product	Etiologic agent	Bacteriophage	Major findings	Country	References
Shrimp	*V. harveyi* Vh10	*Siphoviridae* family phage PhVh6	The bacteriophage infection was optimum at MOI 1 level to kill the host strain. The lytic ability of the bacteriophage was optimum at pH 6 to 8. PhVh6 could tolerate the	Malaysia	(Ibrahim et al., 2017)

			salinities of 15, 30, and 45 ppt level. Overall the authors suggested that the bacteriophage can be used as a biocontrol agent against *Vibrio* spp. in shrimp aquaculture.		
Not reported	*V. harveyi* VHJR7	*Myoviridae* family bacteriophage VhKM4	The bacteriophage was isolated by using *V. harveyi* VHJR7 as a host. It showed wider host infectivity (could infect *V. parahaemolyticus*). This phenomenon suggested that the bacteriophage could be used in different *Vibrio* sp. specific diseases in aquaculture.	Malaysia	(Lal et al., 2017)
Black tiger shrimp	*V. harveyi* VH13-1	*Podoviridae* family bacteriophage VH-P	The temperate *Podoviridae* family bacteriophage was isolated by using a *V. harveyi* isolate VH13-1. In this study, the author proved that at least MOI 20 was needed to kill all of the bacteria in their set up.	Thailand	(Pasharawipas et al., 2011)
Shrimp	*V. harveyi*	*Siphoviridae* family bacteriophage PW2	The bacteriophage showed broad host infectivity against *V. harveyi.* The bacteriophage adsorption rate increased rapidly in the 15 min of infection to 80% and continued to increase by 90% within 30 min of infection. The latent and burst periods of PW2 were 30 and 120 min, respectively, with a burst size of about 78 PFU. The bacteriophage was also stable in the pH in between 4 – 10 and the survival rate at 60 ^{o}C was 50% suggesting its potential use in a broad environment.	Thailand	(Phumkhachorn & Rattanachaikunsopon, 2010)
Tiger shrimp	*V. harveyi* 1114	*Siphoviridae* family bacteriophage VHS1	Genetically the bacteriophage was unique as no other bacteriophage sequence showed significant homology in BLASTN analysis. The bacteriophage can withstand the 60 ^{o}C temperature for 2 h and a wide range of pH for overnight.	Thailand	(Pasharawipas et al., 2005)
Shrimp larvae *Penaeus monodon*	*V. harveyi*	*Myoviridae* family bacteriophage VHLM	Bacteriophage VHML showed a narrow host range and an apparent preference for *V. harveyi* rather than other 63 *Vibrio* isolates and 10 other genera. Later the bacteriophage was sequenced which proved the presence of virulence and lysogenic related genes. Therefore, the use of this bacteriophage as a biocontrol agent is questionable.	Australia	(Oakey et al., 2002; Oakey & Owens, 2000)

NATIONAL STATUS

Vinod et al. (2006) reported that their isolated bacteriophage has the potential to control the population of pathogenic *Vibrio harveyi* in a hatchery setting. In this study, the authors proved that the survivability of *Penaeus monodon* was 86% after treatment of *V. harveyi* and the bacteriophage, whereas it was only 17% in the control (without bacteriophage treatment) and 40% in the antibiotic-treated tanks. A similar type of findings was also observed by Karunasagar et al. (2007). Their results showed that the survival rate of *Penaeus monodon* larvae in bacteriophage treated tank was 86–88% compared to *V. harveyi* treated tank and in the antibiotic-treated tanks the survival rate was 65–68%. Detailing on the effect of bacteriophages in *V. harveyi* infection is tabulated in Table 2. All of these studies concluded that bacteriophages were effective in controlling luminous vibriosis in shrimp culture and sometimes the bacteriophages were more effective than an antibiotic.

Table 2. Potential application of bacteriophage for controlling *Vibrio harveyi* in India.

Aquaculture product	Etiologic agent	Bacteriophage	Major findings	References
Shrimp	*V. harveyi*	Bacteriophage V	The application of recombinant shrimp lysozyme in combination with *V. harveyi* bacteriophage significantly improved the activity of bacteriophage in *in vitro* assay as well as in microcosm study using seawater.	(Choudhury et al., 2019)
Penaeus monodon	*V. harveyi*	*Myoviridae* family bacteriophage VHM1 and VHM2 & *Siphoviridae* family bacteriophage VHS1	The application of the bacteriophage at 10^9 PFU/ml on *Penaeus monodon* larvae infected with *V. harveyi* (10^5 CFU/ml) showed the enhancement of larvae survival after 96 h compared to control. The bacteriophage VHS1 and VHML were sequenced. The presence of the toxin associated with hemocyte agglutination was probably responsible for mortality in the giant tiger shrimp.	(Khemayan et al., 2012; Stalin & Srinivasan, 2017)
Shrimp	*V. harveyi*	*Siphoviridae* family (ΦVh1, ΦVh2, and ΦVh4) and *Podoviridae*	Their major findings were that the isolated bacteriophage could infect 78-98% of *V. harveyi* (125 isolates). The bacteriophages were partially characterized.	(Thiyagarajan et al., 2011)

		family (ΦVh3)		
Shrimp larvae *Penaeus monodon*	*V. harveyi*	*Siphoviridae* family (Viha8 and Viha10) and uncharacterized bacteriophage (Viha9 and) Viha11	Over 85% survival of *Penaeus monodon* larvae after bacteriophage treatment. The normal hatchery practice of antibiotic treatment could only result in 65-68% of survival. The study concluded that bacteriophages could be used for biocontrol of *V. harveyi*.	(Karunasagar et al., 2007)
Shrimp	*V. harveyi*	*Siphoviridae* family (VH1 to VH8)	All of the bacteriophages caused lysis of the host bacterial cells within 2 h. The propagation curve for each bacteriophage shows a burst time started from 1 to 10 h. Isolated bacteriophages of *Vibrio* species could be effective in *in vivo* condition as biological agents to control these pathogenic bacteria in aquaculture systems.	(Srinivasan et al., 2007)
Penaeid shrimp	*V. harveyi*	Bacteriophage specific to *V. harveyi* (Viha1 to Viha7), six from *Siphoviridae* family, and one from *Myoviridae* family (Viha4)	All of the bacteriophages were found to be highly lytic for *V. harveyi* and had different lytic spectrum for a large number of isolates tested. Three of the bacteriophages (Viha1, Viha3, and Viha7) caused 65% of the strains to lyse while Viha2, Viha4, and Viha6 caused 40% of the host strains to lyse. Viha5 had a narrow spectrum (14%). Six of the seven bacteriophages isolates had a broad lytic spectrum and could be potential candidates for biocontrol of *V. harveyi* in aquaculture systems.	(Shivu et al., 2007)

It is clear from the above review of literature that most of the studies on *V. harveyi*-infecting bacteriophages are limited to the isolation and phenotypic characterization; a very few studies aimed to the application of the *V. harveyi*-infecting bacteriophages in shrimp culturing pond. Considering this, the present proposal deals with the isolation and characterization of *V. harveyi*-infecting bacteriophages.

Chapter 3: Aims and Objectives

3. Aims and Objectives

The aim of the study was to use *Vibrio harveyi* infecting bacteriophage as biocontrol agent.The objectives are:

i. Isolation of *Vibrio harveyi*-infecting bacteriophages.

ii. Partial characterization of *Vibrio harveyi* -infecting bacteriophages.

Chapter 4: Materials and Methods

4. Materials and Methods

***4.1. Samples*:** Different types of aquaculture samples (water and soil)were used for *Vibrio harveyi*-infecting bacteriophage isolation.

4.2. Propagation of phages: Ten gram or milliliter of sample was mixed with 20 ml of nutrient broth and 1 ml of each overnight-grown bacterial culture (*V. harveyi* strains) and then incubated at 37 °C with agitation (160 rpm) for 12-18 h. After incubation, samples were centrifuged at 13,000 RPM for 10 min and the supernatant was used for phage isolation. Positive samples (phage containing) were selected based on the dotting assay and the single plaque was isolated.

4.3. Host range analysis*:* Five microliter of virions (1×10^8 PFU [plaque forming units]/ml) was dotted onto the TSA soft agar overlayer containing the test bacteria. The plates were incubated at 37 °C and bacterial growth inhibition was monitored.

***4.4. One-step growth assay*:** A one-step growth assay of phages was performed as previously described Ellis and Delbrück (1939). To summarize, the culture of *V. harveyi* was grown overnight, added to 25 mL of fresh nutrient broth media (1% inoculum) and incubated at 28°C. When the optical density at 600 nm (OD_{600}) will be reached at 0.3 (approximately 1×10^7 CFU/ml), the bacteriophage was added at MOI of 0.01 and incubated for 5 min, followed by centrifugation at 8000 rpm for 10 min. The pellet was resuspended in the samevolume of fresh nutrient broth. Then 1 mL of this mixture was aliquoted into micro- centrifuge tubes (1.5 ml) followed by incubation at 37°C with shaking (160 rpm). At every 10 min, one tube was taken out from the incubator and centrifuged at 8000 rpm for 3 min. The supernatant was then collected and used for PFU counting by using TA soft agar.

***4.5. Liquid culture inhibition assay*:** Growth inhibition of *E. coli* MTCC 433 by bacteriophage in liquid culture was performed as described by Shin et al. (2011) with some modification. An overnight culture of *V. harveyi* (1% inoculum) was added to fresh nutrient broth media and incubated at 37 °C with shaking (160 rpm). After 3 h when OD_{600} was reached approximately 0.3, bacteriophage was added at MOI of 1 and 0.1 levels. The samples were collected at every 1 h and OD_{600} was measured using spectrophotometer.

4.6 ***Sability testing*:** The bacteriophages' stability were tested at broad pH range (pH 5-9), high alkanity, and broad salinity (0-40 ppt) by the standard methods (Ibrahim et al., 2017). Briefly, SM buffer was calibrated using 1 M HCl to pH 3, 5, 6, 7, 8, and 9 and 900 µl of each calibrated solution was aliquoted into 1.5 ml vials. The bacteriophage stock was added to the calibrated buffer solutions to the final titer of 10^8 PFU/ ml. The vials was gently inverted to

mix, and left to stand at room temperature for 60 min. After 60 min, 100 µl of each solution were sampled and mixed with 100 µl of exponentially growing *E. coli*. After 12 h incubation at 35 °C, the pH resulted in the highest number of plaque (titer) was considered optimum.

4.7 ***Statistical analysis:*** All of the experiments were carried out three times. The data are represented as the mean ± Standard Deviation (SD).

Chapter 5: Results and Discussion

5. Results and Discussion

5.1. Isolation of bacteriophage

Bacteriophage VP3 was isolated from an environmental sample by using *V. harveyi* as a host. This bacteriophage showed clear plaques after 12 hours incubation at 37°C. Plaquewas purified three times after isolation.

5.2. Host range analysis of bacteriophage

For host range analysis VP3 *Vibrio parahemolyticus, Vibrio harveyi* ATCC 14126, *Lactobacillus* sp, *Aeromonas hydrophila* MTCC 1739 were used as hosts. The phage could infect *Vibrio parahemolyticus, Vibrio harveyi ATCC 14126*, whereas, it could not infect the other tested strains (Table 1).

Table 1. Host range analysis of isolated phage.

Phage name	Bacteria name			
	Vibrio parahemolyticus	*Vibrioharveyi* ATCC 14126	*Aeromonas hydrophila*MTCC 1739	*Lactobacillus* sp.
VP3	+	+	-	-

5.3. One step growth curve of bacteriophage VP3

In one step growth curve, the latent period of the phage VP3 was 20 min and the one step cycle was completed at 50 min (Fig.1). The burst size was 33 on propagation strain *V. harveyi* S3A. The latent period of VP3 was comparable to the *Vibrio*-infecting bacteriophages such as VP11 10 min which was Short latent period. However, the burst sizes of this bacteriophages 17 were found to be different from bacteriophage VP3 (Tan et al., 2021).

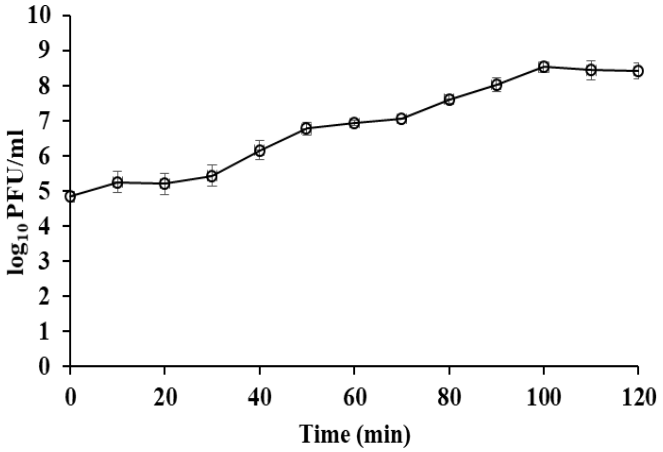

Fig. 1. One step growth curve of *V. harveyi* bacteriophage VP3.

5.4. Liquid culture inhibition of bacteriophage VP3

E. coli infecting bacteriophage VP3 effectively inhibited the growth of *V. harveyi* S3A in liquid culture. Clear lysis was observed after 6 h of post infection for MOI 0.1 and MOI 1 whereas the OD_{600} of the control (no bacteriophage addition) was 1.432 after 6 h (Fig. 2). Similar lysis patterns of *V. harveyi* phages were also observed (Tan et el., 2021). As the phage could clearly lysed its host bacteria, the phage might have lytic activity and it could be used as biocontrol agent.

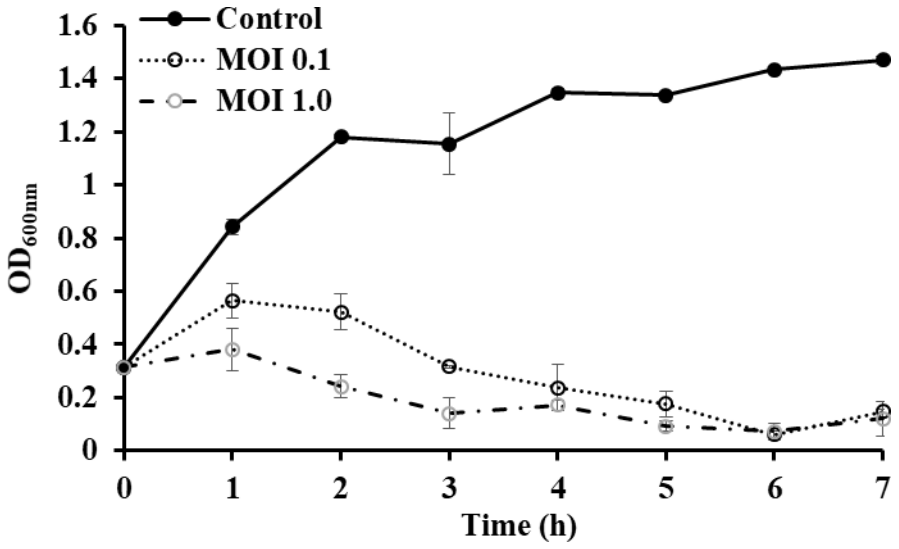

Fig. 2. Liquid culture inhibition of *V. harveyi*-infecting bacteriophage VP3.

5.5. pH stability of bacteriophage VP3

The sensitivity of *V. harveyi*- infecting bacteriophage VP3 at different pH was shown in Fig. 3.The survival rates at pH 3, 5, 6, 7, 8, and 9 were 97.22%, 97.82%, 98.27%, 99.06%, 99.20% and 98.69% after 1 h, respectively. Similarly, 91.75%, 92.84%, 93.56% 94.21%, 97.61%, and 95.84% survivability after 6h; and 89.24%, 89.24%, 92.65%, 93.86%, 94.14% and 91.59% survivability were observed after 24 h. for pH 3, 5, 6, 7, 8, and 9, respectively (Fig. 3). Ibrahim et al. (2017) also observed that their isolated phages were also stable at broad pH range. Hence, it is clearthat the phage could survive in extreme acidic and alkaline condition.

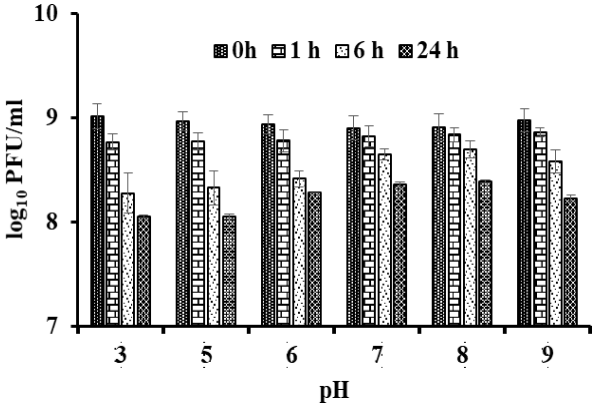

Fig. 3. pH stability of *V. harveyi*-infecting bacteriophage VP3.

5.6. Stability of bacteriophage VP3 to temperature

Bacteriophage VP3 survivability were decreased as temperature dependent manner. The survival rates of bacteriophage VP3 after 30 min were 99.26%, 98.58%, 96.89%, 80.14%, after 60 min 98.68%, 98.45%, 95.70%, 57.01% after 90 min 98.70%, 98.25%, 94.68%, 29.26% and after 120 min 99.16%, 98.08%, 94.00%, and 21.58% at 4º, 28º, 37º, 60º C respectively (Fig. 4). The *E. coli* bacteriophage PhVh6 was also found to be stable at 4º to 37º C temperature (Ibrahim et al., 2021).

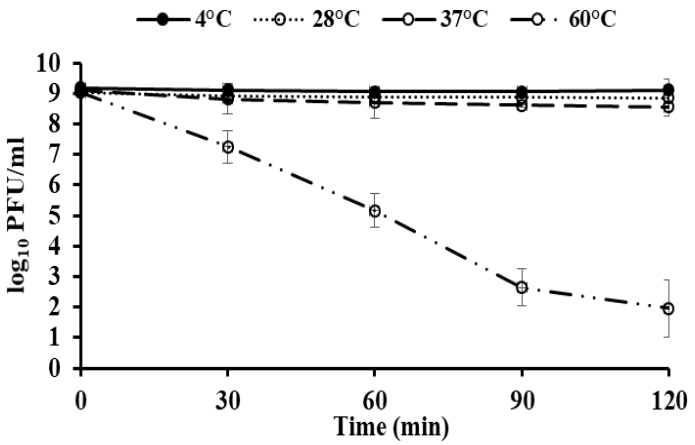

Fig. 4. Temperature stability of *V. harveyi* bacteriophage VP3.

Chapter 6: Conclusion

6. Conclusion

In this study, we have isolated a *V. harveyi*-infecting bacteriophage from the aquaculture water. The isolated bacteriophage VP3 could lyse *V. harveyi* but unable to infect other tested bacteria.. The bacteriophage also proved its ability to inhibit the growth of *V. harveyi* in liquid culture and had broad pH and temperature stability. Moreover, the bacteriophage successfully inhibited the growth of *V. harveyi* in aquaculture environment. Hence, it can be concluded that the bacteriophage might be used as a biocontrol agent. Clearly, a detailed study is needed to prove its lytic ability in different environmental condition.

7. Future Scope

7. Future Scope

There is an enough scope to prove the ability of the isolated bacteriophage to control *V. harveyi* in different environment. Moreover, the genome annotation of the bacteriophage would enrich the classification scheme of the *V. harveyi*-infecting bacteriophages. Furthermore, immobilization of the bacteriophages may help to increase their viability.

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