Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 1393 – 1407 (2025)
www.ejabf.journals.ekb.eg

Host Range of Lytic Bacteriophages as Biocontrol Agents for Pathogenic Bacteria Causing Foodborne Illnesses in the Vannamei Shrimp (*Litopenaeus vannamei*)

Ananda Sekar Kinanti[1*], Asep Awaludin Prihanto[2], Yoga Dwi Jatmiko[3]

[1]Master Study Program, Department of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, JL. Veteran, Malang 65415, East Java, Indonesia
[2]Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University, JL. Veteran, Malang 65415, East Java, Indonesia
[3]Department of Biology, Faculty of Fisheries and Marine Science, Brawijaya University, JL. Veteran, Malang 65415, East Java, Indonesia
[*]**Corresponding Author: anandasekar@student.ub.ac.id**

ARTICLE INFO

Article History:
Received: Dec. 18, 2024
Accepted: Jan. 28, 2025
Online: Feb. 7, 2025

Keywords:
Vibrio parahaemolyticus,
Bacteriophage,
Shrimp quality,
Antibiotic resistance,
Foodborne illness

ABSTRACT

Ensuring the quality of shrimp is essential to prevent exposure to *Vibrio* sp. bacteria. Consuming seafood contaminated with these bacteria can cause food poisoning. *Vibrio parahaemolyticus* is a bacterium responsible for countless cases of foodborne illness. Prolonged use of antibiotics to treat these infections may lead to antibiotic resistance. As a safer alternative, bacteriophages can be used to combat *Vibrio parahaemolyticus* infections. Bacteriophages are viruses that specifically target bacteria. This study aimed to isolate bacteriophages from blood clams and green mussels. Bacteriophages isolated from blood clams (*Anadara granosa*) and green mussels (*Perna viridis*) were effective in lysing the bacteria, as demonstrated by plaque formation on double-layer agar plates. The bacteriophage density from blood clams was 3.7×10^4 PFU/mL, while from green mussels, it was 1.38×10^7 PFU/mL. Host range tests showed that both bacteriophages were capable of lysing *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio harveyi*, and *Aeromonas hydrophila*.

INTRODUCTION

Vibrio parahaemolyticus is currently one of Indonesia's leading export commodities, as seen from its potential market prospects. However, shrimp exports have problems with food quality and safety. One of the shrimp quality requirements according to SNI 01-2728.1-2006 is that it is free from *Vibrio* sp. bacteria contamination. Consuming seafood that has been contaminated with bacteria or the toxins it produces can cause food poisoning (foodborne disease). The incidence of foodborne diseases after consuming seafood is 10-20% of cases caused by *Vibrio* spp. bacteria **(Hikmawati *et al.*, 2019)**. The Food and Environmental Hygiene Department reported that 28.7% of the 313 cases of food poisoning in Hong Kong from 1999 to 2003 were due to the consumption

of crustaceans including shrimp, lobster, and others caused by *Vibrio* **(Ahmed & Amin, 2018)**.

Vibrio parahaemolyticus is one of the *Vibrio* species that is pathogenic to both shrimp commodities and humans. Research conducted by **Fasulkova and Stratev (2024)** explains that *Vibrio parahaemolyticus* has become a leading cause of seafood-associated gastroenteritis in Japan, the United States, and various other parts of the world. Acute infections can lead to skin infections, gastroenteritis, and even death **(Negrut *et al.*, 2020)**. Efforts to combat foodborne diseases caused by *Vibrio parahaemolyticus* often involve the use of antibiotics, which are commonly consumed and used as a treatment for infectious diseases. However, continuous use of antibiotics can inadvertently lead to resistance in pathogenic bacteria **(Bakkeren *et al.*, 2020; Huemer *et al.*, 2020)**. One relatively safe approach to addressing *Vibrio parahaemolyticus* and managing foodborne diseases is through the use of bacteriophages **(Garvey, 2022; Lavilla *et al.*, 2023)**.

Bacteriophages or bacterial viruses are obligate intracellular parasites that can only reproduce by exploiting bacterial cells as their host, making them suitable as bacterial biocontrol agents. Bacteriophages can be sourced from samples that match the habitat of the target bacteria **(Naureen *et al.*, 2020; Guererro *et al.*, 2021)**. Research by **Gyawali and Hewitt (2020)** reported that *Anadara* spp. clams from the Gulf of Nicoya, Costa Rica, were contaminated with *Escherichia coli*, *Salmonella* spp., and *Vibrio parahaemolyticus*. Additionally, **Lubis *et al.* (2022)** stated that green mussels were contaminated by *Vibrio cholerae* and *Vibrio parahaemolyticus*. As filter feeders, clams filter water to obtain food, making them susceptible to contamination by microorganisms such as *Vibrio parahaemolyticus* **(Abd Wahid *et al.*, 2022)**. Therefore, research on blood clams (*Anadara granosa*) and green mussels (*Perna viridis*) as reservoirs for bacteriophages is necessary.

MATERIALS AND METHODS

This research samples of blood mussels (*Anadara granosa*) and green mussels (*Perna viridis*) were collected from the Mangrove Center Banyuurip, Ujungpangkah, Gresik, East Java. The green mussel (*Perna viridis*) samples were harvested from the sea using ropes tied to bamboo, while the blood mussel (*Anadara granosa*) samples were manually collected during low tide. The samples were then placed in an insulation box containing crushed ice and transported to the Advance Laboratory, Faculty of Fisheries and Marine Science, Brawijaya University.

Preparation of *Vibrio parahaemolyticus* bacteria

The *V. parahaemolyticus* isolate, used as the host bacterium for confirmation, was cultured on selective media, specifically TCBS (Thiosulfate Citrate Bile Salts Sucrose Agar). A loopful of stock culture was taken and inoculated on TCBS medium using the

streak plate method and incubated at 37°C for 24 hours **(Lee *et al.*, 2022)**. The growth of *V. parahaemolyticus* on TCBS medium was indicated by small, greenish-blue colonies **(Pawar & Salgaonkar, 2023)**. Further confirmation was carried out by observing the cell morphology of *V. parahaemolyticus* microscopically through Gram staining.

Bacteriophage isolation

The bacteriophage isolation process was conducted according to the method of **Tan *et al.* (2021)**, with modifications. In this process, 25g of shrimp shell samples and 25mL of pond wastewater were placed into separate Erlenmeyer flasks, and then 225mL of SM Buffer was added. The mixture was centrifuged at 10,000 rpm at 4°C for 20 minutes. Subsequently, 20mL of the supernatant resulting from centrifugation was mixed with 10mL of TSB media and 5mL of *Vibrio* sp. bacteria, followed by incubation for 24 hours at 37°C. The phage suspension produced was centrifuged at 4°C for 10 minutes at 10,000rpm. The resulting supernatant was then filtered using a 0.22µm membrane filter. The phage suspension produced was detected using plaque assay method and double-layer technique. The obtained bacteriophage was then characterized for its morphology using transmission electron microscopy (TEM).

Determination of bacteriophage density

According to **Jatmiko *et al.* (2018)**, bacteriophage density was determined using a serial dilution method. A bacteriophage stock was prepared, and 0.1ml was added to 0.9ml of SM Buffer. Dilutions were performed up to 10^{-10}, with each dilution consisting of 100µl of the bacteriophage solution, which was then added to 3ml of TSA medium (0.6%) containing 100µl of *Vibrio parahaemolyticus* and homogenized. The suspension was poured into separate petri dishes containing TSA 1.5% and incubated at 37°C for 48 hours. Plaques that met the criteria (within a range of 25-250 plaques) were observed and counted. If a lower bacteriophage density was expected, the bacteriophage stock was further diluted with SM Buffer. The formula for calculating bacteriophage density was as follows.

$$\text{Total Plaque} = \frac{\text{Number of plaques}}{\text{Lysate volume x Dilution factor}}$$

Host range

The host range test, based on the method of **Jatmiko *et al.* (2018)**, was conducted using the spot test method. Host cultures were prepared in the early log phase in Nutrient Broth (NB). The host bacteria used were *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Vibrio harveyi*, *Vibrio cholerae*, and *Vibrio alginolyticus*. For each host culture, 0.1ml of bacteria in the early log phase was taken and spread onto the surface of Tryptic Soy Agar (TSA) using a cotton swab, then left at room temperature for 30 minutes. Next, 5µl of bacteriophage stock was inoculated

onto the TSA media with the host bacteria and incubated at 37°C for 24 hours to allow the formation of plaques or clear zones.

RESULTS

Bacteria *Vibrio parahaemolyticus*

Colonies from TCBS plates were selected for further identification and isolated by loop. Further confirmation was done by microscopic morphology of *V. parahaemolyticus* cells through Gram staining, as shown in Fig. (1).

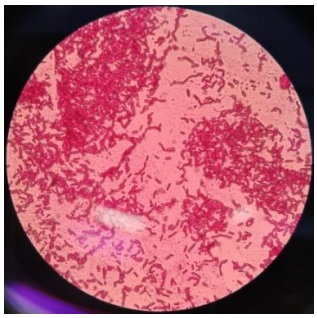

Fig. 1. Gram staining of *Vibrio parahaemolyticus* bacteria

Phage isolation

Blood clam (*Anadara granosa*) and green mussels (*Perna viridis*) serve as samples capable of producing bacteriophages. Successful bacteriophage isolation is indicated by the formation of plaques on double-layer agar plates. A plaque is a clear, round area that shows bacterial cell lysis caused by the phage. The results of bacteriophage isolation show plaque formation in *Vibrio parahaemolyticus* cultures derived from blood clam and green mussel samples, as presented in Fig. (2).

Bacteriophages constitute a group of viruses that can specifically infect and lyses bacteria **(Wegrzyn, 2022)**. Phages could prove to be superior to antibiotics, since they are persisting, inactive and non-pathogenic outside their bacterial hosts. Compared with other conventional therapeutic approaches, phage therapy could be more effective in treating emerging resistant pathogenic bacterial strains. It is easily detectable, can kill biofilm-forming bacteria, and has low inherent toxicity. Phages during the lytic course are capable of increasing in number specifically where hosts are located **(Bono *et al.*, 2021)**. Generally, it does not affect beneficial bacteria, and side effects are uncommon in phages, as they do not impact eukaryotic cells.

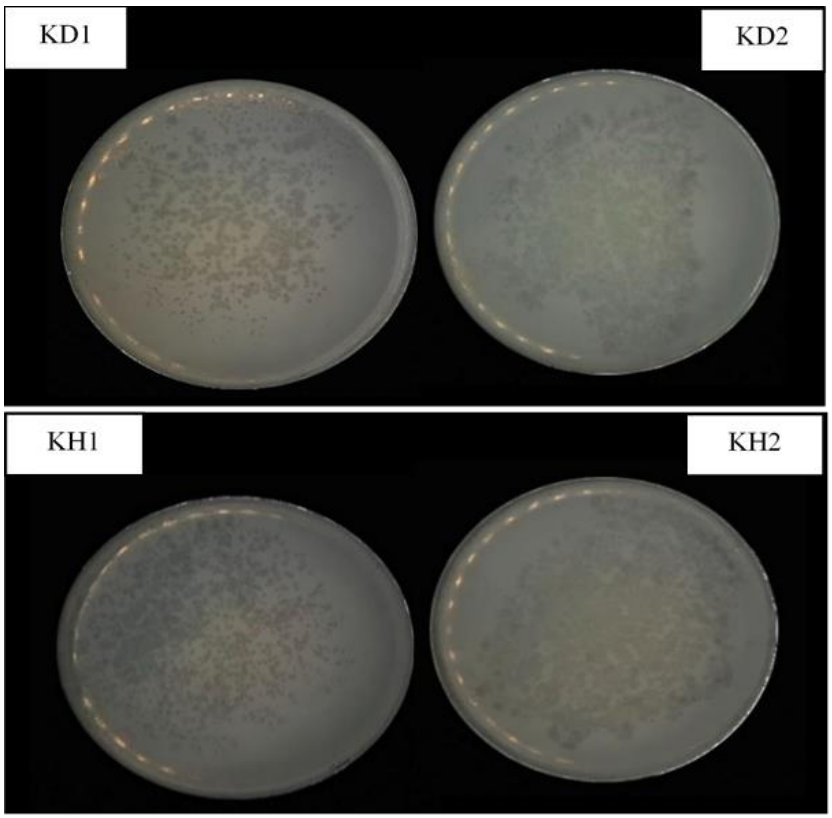

Fig. 2. Isolation of bacteriophages (KD: Blood Clam; KH: Green Clam) was repeated twice

Table 1. Size of the isolated plaques from samples

Sampling site	Plaque size	Plaque morphology
Phage -KD1	1 mm	Clear zone
Phage -KD2	5 mm	Clear zone
Phage -KH1	2 mm	Clear zone
Phage -KH2	7 mm	Clear zone

Note: KD) Blood clam; KH) Green mussel

Determination of bacteriophage density

The highest bacteriophage isolate density was found in green mussels (1.38×10^7 PFU/mL), while blood clams had a density of 3.7×10^4 PFU/mL. This was also observed from the plaques present in the KD isolate, which was diluted up to 10^6, whereas the KH isolate was only diluted up to 10^3. The density results from this study are illustrated in Fig. (3).

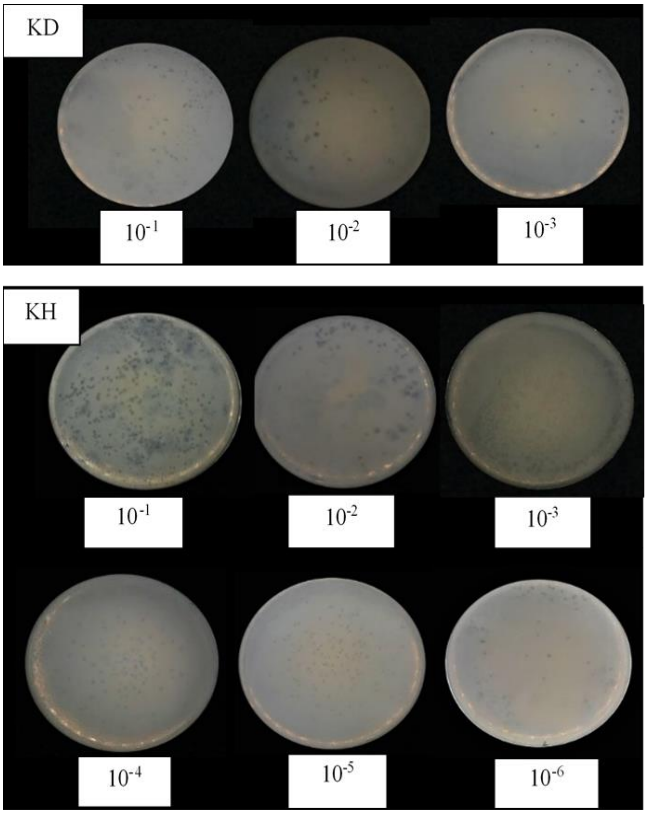

Fig. 3. Bacteriophage density results; KD) Blood clam; KH) Green mussel

The variation in bacteriophage titer at each dilution is caused by the bacterial defense system against bacteriophage infection, the properties of the bacteriophages, and the dilution treatments. This is consistent with the research by **Ranveer *et al.* (2024)**, which indicates that the inability of bacteriophages to lyse their hosts can occur due to differences in environmental conditions, faster host growth, and natural defense mechanisms against bacteriophage infection. The number of plaques is also influenced by the specificity of the bacteria itself. Viruses that cannot infect bacteria are due to the bacteriophage particles produced during infection having several imperfect components **(Lauman & Dennis, 2021; Brachelente *et al.*, 2023)**. From the results of the bacteriophage density, calculations were carried out by following the plaque requirements of 25-250. The results of the bacteriophage density calculation can be seen in Table (2).

Host range bacteriophage

Each type of virus can infect cells from a limited range of hosts, referred to as the host range. Host range testing of the bacteriophage isolates indicates that blood clams and green mussels are capable of lysing bacteria other than *Vibrio parahaemolyticus* (Fig. 4).

Table 2. Results of bacteriophage density

No.	Dilution factor	Plaque count			
		KD	Density	KH	Density
1.	10^{-1}	110	$1{,}1x10^{4}$	240	$2{,}4x10^{4}$
2.	10^{-2}	63	$6{,}3x10^{4}$	215	$2{,}15x10^{5}$
3.	10^{-3}	20	-	160	$1{,}6x10^{6}$
4.	10^{-4}	0	-	136	$1{,}36x10^{7}$
5.	10^{-5}	0	-	54	$5{,}4x10^{7}$
6.	10^{-6}	0	-	20	-
7.	10^{-7}	0	-	8	-
8.	10^{-8}	0	-	0	-
9.	10^{-9}	0	-	0	-
10	10^{-10}	0	-	0	-
Average Density (PFU/mL)			**$3{,}7x10^{4}$**		**$1{,}38x10^{7}$**

Note: KD) Blood clam, KH) Green mussel

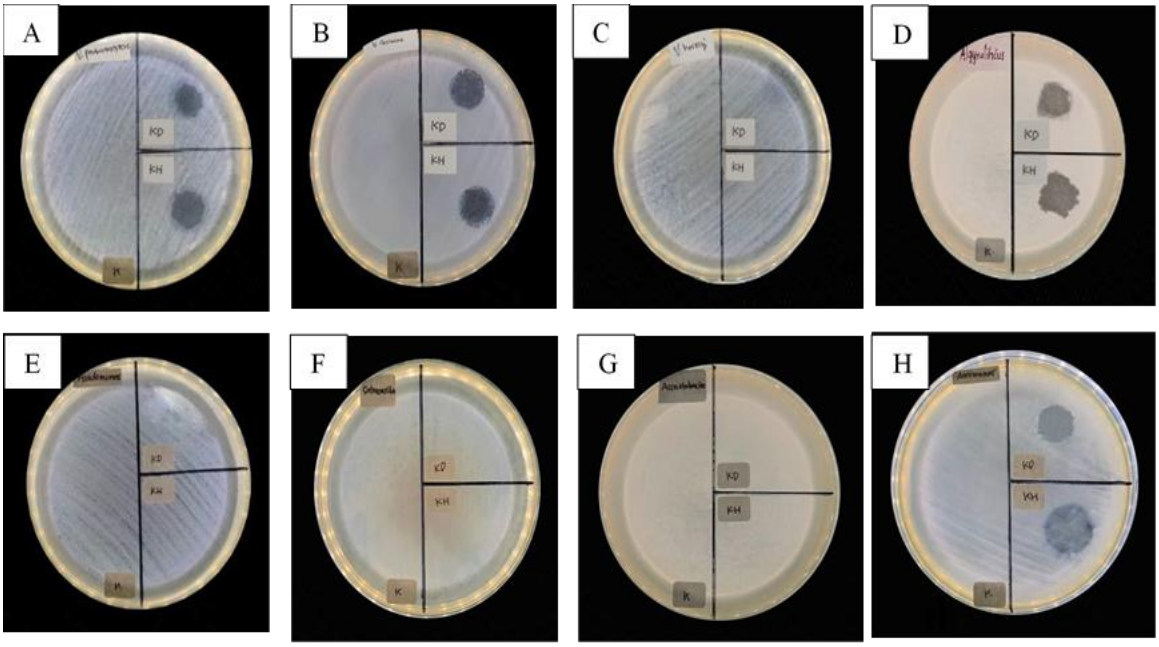

Fig. 4. A) *V. parahaemolyticus*; B) *V. cholerae*; C) *V. harveyi*; D) *V. alginolyticus*; E) *Pseudomonas aeruginosa*; F) *Salmonella typi*; G) *Acinetobacter baumannii*; H) *Aeromonas hydrophila*

Several factors prevent phages from infecting other bacterial strains, including variations or differences in host cell receptor molecules (adsorption blocking), the host cell's restriction-modification system, and phage resistance mechanisms that result in failure to infect the host cell **(Jurado *et al.*, 2022)**. Additional factors, such as the host's restriction-modification system and resistance mechanisms, also contribute to the phage's inability to infect other bacterial strains. Adaptations to different environmental conditions and exploitation of different vectors are influential as well. Phages can significantly modify bacterial communities, depending on their potential to propagate within those communities **(Thung *et al.*, 2017; Drew *et al.*, 2021)**.

Table 3. Results of host range bacteriophage

No.	Host bacteria	Isolate code	
		KD	**KH**
1.	*Vibrio parahaemolyticus*	+	+
2.	*Vibrio cholerae*	+	+
3.	*Vibrio alginolyticus*	+	+
4.	*Vibrio harveyi*	-	-
5.	*Salmonella typi*	-	-
6.	*Aeromonas hyrophila*	+	+
7.	*Pseudomonas aeruginosa*	+	-
8.	*Acinetobacter baumannii*	-	-

Note: KD) Blood clam, KH) Green mussel

Morphology bacteriophage

Bacteriophage characterization was performed using the transmission electron microscopy (TEM). The morphological characterization of phage strains on phage code, tail type, and family can be found in Table (4).

Table 4. Characterization of bacteriophage

Phage code	Morphology		
	Plaque	**Tail type**	**Family**
KD1	Medium size	Non-contractile	*Tectiviridae*
KD2	Medium size	Non-contractile	*Tectiviridae*
KH1	Small	Non-contractile	*Cystoviridae*
KH2	Small	Non-contractile	*Cystoviridae*

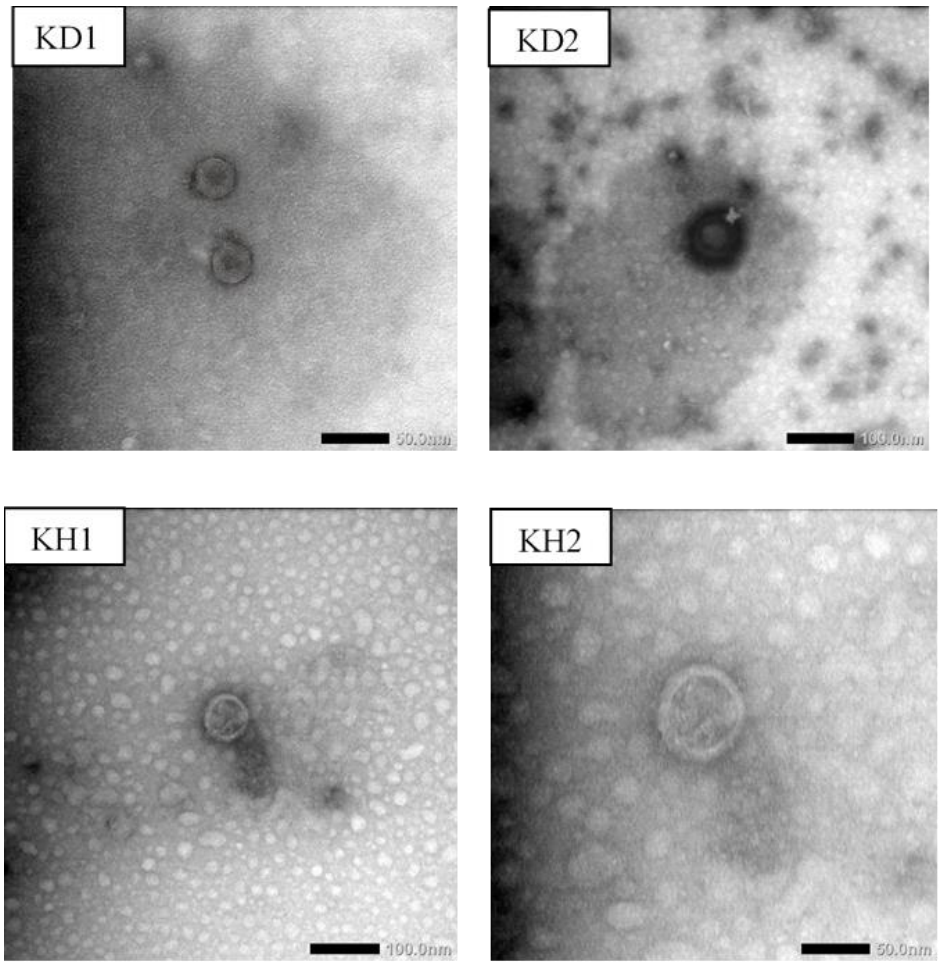

Fig. 5. Bacteriophage density results; KD) Blood clam; KH) Green mussel

DISCUSSION

The Gram staining results showed that the bacterial isolate was rod-shaped and classified as Gram-negative because it produced red-colored colonies after Gram staining. This is consistent with the statement of **Alawiyyah *et al*. (2017)**, which describes *Vibrio parahaemolyticus* as having pink-colored cells, a characteristic of Gram-negative bacteria, with a rod shape and occurring as single cells. According to **Lainjong (2024)**, Gram-negative bacteria, after being stained with a primary dye, lose their color when treated with alcohol and then absorb safranin as a counterstain, appearing red (the color of safranin) on the slide. In contrast, Gram-positive bacteria retain the primary dye and are not decolorized by alcohol, appearing violet due to the crystal violet stain and do not absorb the counterstain.

The formation of plaques on agar plates indicates bacterial cell lysis caused by bacteriophages. Plaques are categorized into two types: clear plaques and cloudy plaques.

Clear plaques indicate lytic phages, while cloudy plaques suggest lysogenic phages **(Jing, 2012)**. According to **Dennehy and Abedon (2021)**, virulent phages lead to rapid lysis and the death of the bacterial host cell, whereas temperate (lysogenic) phages spend part of their life cycle in a dormant state known as a prophage. One factor influencing plaque characteristics is the adsorption rate of viral genetic material into the host cell. Additionally, plaque size and quantity can vary due to several factors, including the specific bacteriophage species, as each species produces a different number of progeny **(Glonti & Pirnay, 2022)**.

The highest bacteriophage density was obtained from the green mussel samples. This is influenced by various environmental factors, including industrial waste, household waste, and other food residues. Such contamination can trigger the growth of *Vibrio* sp. and expose the biota in a given water body **(Lubis *et al.*, 2022)**. Bacteriophage density can also be affected by differences in the viability of bacteriophages in each sample. Poor environmental conditions (acidity, temperature, and moisture content), along with the transition of the host into a dormant phase, can reduce bacteriophage productivity **(Lukman *et al.*, 2020; Wdowiak *et al.*, 2022)**.

Based on the host range testing results, two bacteriophage isolates exhibited a narrow host range against *Vibrio harveyi*, *Salmonella typhi*, and *Acinetobacter baumannii*. This narrow host range is attributed to adaptations to different environments and the host's resistance systems against phage infection **(Chevallereau *et al.*, 2022; Huang *et al.*, 2024)**, which means that phages with a narrow host range can only lyse a limited number of host bacterial strains. The results of the host range tests were more specific to *Vibrio cholerae*, *Vibrio alginolyticus*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*. Bacteriophages with a wide host range can lyse many strains of host bacteria, making them widely used for phage applications **(Sliwka *et al.*, 2022; Bozidis *et al.*, 2024; Kovacs *et al.*, 2024)**.

The Tectiviridae family is characterized by bacteriophages that have non-contractile tails **(Evseev *et al.*, 2024)**. These phages have a unique structure with an icosahedral capsid containing an internal lipid membrane, but they do not possess a tail structure that contracts. The tail is typically short and rigid, used for attachment to the bacterial host but not for injection through a contractile mechanism. The icosahedral capsids of Tectiviridae members are about 65-70 nanometers in diameter, placing them in the small to moderate size range **(Sanz-Gaitero, 2021)**.

The Cystoviridae family of bacteriophages is characterized by having non-contractile tails. These phages are unique in that they have an enveloped structure with a segmented double-stranded RNA genome. The Cystoviridae family is generally classified as small to moderate in size. These bacteriophages have a diameter of approximately 80-100 nanometers, which places them in the small to medium size range compared to other bacteriophages **(Salomaa, 2021)**. Their structure includes an icosahedral capsid with an

envelope, which contributes to their slightly larger size compared to non-enveloped small phages.

CONCLUSION

Bacteriophages targeting *Vibrio parahaemolyticus* were successfully isolated from green mussels and blood clams, demonstrating a broad host range against *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio harveyi*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*. The bacteriophage isolated from blood clams was classified under the order Petitvirales within the Tectiviridae family, while the bacteriophage from green mussel was classified under the order Mindivirales within the Cystoviridae family. Further purification and molecular identification of the isolated bacteriophages are necessary. Given their demonstrated broad host range against foodborne pathogens, these bacteriophages have potential for development as safer biocontrol agents.

REFERENCES

Abd Wahid, M. E.; Mohamad, M.; Mohamed, N. N. and Afiqah-Aleng, N. (2022). Vibriosis in green mussels. In *Aquaculture Pathophysiology* (pp. 515-529). Academic Press.

Ahmed, O. and Amin, H. (2018). Detection and survival of *Vibrio* species in shrimp (*Penaeus indicus*) and mussel (*Mytilus galloprovincialis*) at landing and after processing at seafood markets in Suez, Egypt. *Journal of Food and Dairy Sciences*, *9*(12), 411–417. **DOI: 10.21608/jfds.2018.36107**

Alawiyyah, T.; Anto, B. and Agung, S. (2017). Isolation, enumeration and molecular detection of ToxR gene in *Vibrio parahaemolyticus* bacteria from vannamae shrimp ponds in Rembang, *Journal of Biology*, 6(3), 96-102.

Bakkeren, E.; Diard, M. and Hardt, W. D. (2020). Evolutionary causes and consequences of bacterial antibiotic persistence. *Nature Reviews Microbiology*, *18*(9), 479-490. **DOI: 10.1038/s41579-020-0378-z**

Bono, L. M.; Mao, S.; Done, R. E.; Okamoto, K. W.; Chan, B. K. and Turner, P. E. (2021). Advancing phage therapy through the lens of virus host-breadth and emergence potential. *Advances in Virus Research*, *111*, 63-110. **DOI: 10.1016/bs.aivir.2021.07.004**

Bozidis, P.; Markou, E.; Gouni, A. and Gartzonika, K. (2024). Does Phage Therapy Need a Pan-Phage?. *Pathogens*, *13*(6), 522. **DOI: 10.3390/pathogens13060522**

Brachelente, S.; Galli, A. and Cervelli, T. (2023). Yeast and Virus-like Particles: A Perfect or Imperfect Couple?. *Applied Microbiology*, *3*(3), 805-825. **DOI: 10.3390/applmicrobiol3030056**

Chevallereau, A.; Pons, B. J.; van Houte, S. and Westra, E. R. (2022). Interactions between bacterial and phage communities in natural environments. *Nature Reviews Microbiology*, *20*(1), 49-62. **DOI: 10.1016/bs.aivir.2021.07.004**

Dennehy, J. J. and Abedon, S. T. (2021). Phage infection and lysis. *Bacteriophages: biology, technology, therapy*, 341-383.

Drew, G. C.; Stevens, E. J. and King, K. C. (2021). Microbial evolution and transitions along the parasite-mutualist continuum. *Nature Reviews Microbiology*, *19*(10), 623-638. **DOI: 10.1038/s41579-021-00550-7**

Evseev, P.; Gutnik, D.; Evpak, A.; Kasimova, A. and Miroshnikov, K. (2024). Origin, Evolution and Diversity of φ29-like Phages-Review and Bioinformatic Analysis. *International Journal of Molecular Sciences*, *25*(19), 10838. **DOI: 10.3390/ijms251910838**

Fasulkova, R., and Stratev, D. (2024). Insights into foodborne *Vibrio parahaemolyticus*–a review. *Food Research*, *8*(1), 190-209. **DOI: 10.26656/fr.2017.8(1).267**

Garvey, M. (2022). Bacteriophages and food production: Biocontrol and bio-preservation options for food safety. *Antibiotics*, *11*(10), 1324. **DOI: 10.3390/antibiotics11101324.**

Glonti, T. and Pirnay, J. P. (2022). In vitro techniques and measurements of phage characteristics that are important for phage therapy success. *Viruses*, *14*(7), 1490. **DOI: 10.3390/v14071490**

Guerrero, L. D.; Pérez, M. V.; Orellana, E.; Piuri, M.; Quiroga, C. and Erijman, L. (2021). Long-run bacteria-phage coexistence dynamics under natural habitat conditions in an environmental biotechnology system. *The ISME journal*, *15*(3), 636-648. **DOI: 10.1038/s41396-020-00802-z**

Gyawali, P. and Hewitt, J. (2020). Faecal contamination in bivalve molluscan shellfish:

can the application of the microbial source tracking method minimise public health risks?. *Current Opinion in Environmental Science & Health*, *16*, 14-21. **DOI: 10.1016/j.coesh.2020.02.005**

Huang, D.; Xia, R.; Chen, C.; Liao, J.; Chen, L.; Wang, D. and Yu, P. (2024). Adaptive strategies and ecological roles of phages in habitats under physicochemical stress. *Trends in Microbiology*. **DOI: 10.1016/j.tim.2024.02.002**

Huemer, M.; Mairpady Shambat, S.; Brugger, S. D. and Zinkernagel, A. S. (2020). Antibiotic resistance and persistence implications for human health and treatment perspectives. *EMBO reports*, *21*(12), e51034. **DOI: 10.15252/embr.202051034**

Hikmawati, F.; Susilowati, A. and Ratna Setyaningsih. (2019). Colony morphology and molecular identification of *Vibrio* spp. on green mussels (*Perna viridis*) in Yogyakarta, Indonesia tourism beach areas. *Biodiversitas Journal of Biological Diversity*, *20*(10), 2891–2899. **DOI: 10.13057/biodiv/d201015**

Jatmiko, Y. D.; Agung, P. P. and Tri, A. (2018). Test of lytic bacteriophage activity from household waste against *Salmonella Typhi*. Biodjati Journal, 3(2), 135-147. **DOI: 10.15575/biodjati.3i2.3471**

Jurado, A.; Fernández, L.; Rodríguez, A. and García, P. (2022). Understanding the mechanisms that drive phage resistance in *Staphylococci* to prevent phage therapy failure. *Viruses*, *14*(5), 1061. **DOI: 10.3390/v14051061**

Kovacs, C. J.; Antonacci, A.; Graham, A.; Jessup, F.; Rankin, W.; Brasko, B. and Barnhill, J. C. (2024). Comparing Methods to Genetically Engineer Bacteriophage and Increase Host Range. *Military Medicine*. **DOI: 10.1093/milmed/usae226**

Lainjong, E. (2024). Identification of Air Bacteria Using Gram Style Methods in The Integrated Laboratory of Bina Mandiri University Gorontalo. *West Science Interdisciplinary Studies*, *2*(02), 485-494. **DOI: 10.58812/wsis.v2i02.700**

Lauman, P. and Dennis, J. J. (2021). Advances in phage therapy: targeting the Burkholderia cepacia complex. *Viruses*, *13*(7), 1331. **DOI:10.3390/v13071331**

Lavilla, M.; Domingo-Calap, P.; Sevilla-Navarro, S. and Lasagabaster, A. (2023). Natural killers: opportunities and challenges for the use of bacteriophages in microbial food safety from the one health perspective. *Foods*, *12*(3), 552.

DOI:10.3390/foods12030552

Lee, J. H.; Park, S. K.; Khan, F.; Jo, D. M.; Lee, D. H., Kang, M. G. and Kim, Y. M. (2022). Simultaneous isolation and enumeration of virulent *Vibrio cholerae* and *Vibrio vulnificus* using an advanced MPN-PCR method. *Archives of Microbiology*, *204*(1). **DOI: 10.1007/s00203-021-02613-y**

Lubis, S. S.; Arif, S. and Sailul, M. (2022). Enumeration and Pathogenicity Test of Vibrio sp. in Green Mussels (*Perna viridis*) from the Krueng Cut Area, Aceh Besar. *Kenanga Journal of Biological*. **DOI: 10.22373/kenanga.v2i1.1920**

Lukman, C.; Yonathan, C.; Magdalena, S. and Waturangi, D.E. (2020). Isolation and characterization of pathogenic *Escherichia coli* bacteriophages from chicken and beef offal. *BMC Research Notes*, *13*(1), 1–7. **DOI: 10.1186/s13104-019-4859-y**

Moller, A. G.; Winston, K.; Ji, S., Wang, J.; Hargita Davis, M. N.; Solis-Lemus, C. R. and Read, T. D. (2021). Genes influencing phage host range in *Staphylococcus aureus* on a species-wide scale. *Msphere*, 6(1), 10-1128. **DOI: 10.1128/msphere.01263-20**

Naureen, Z.; Dautaj, A.; Anpilogov, K.; Camilleri, G.; Dhuli, K.; Tanzi, B. and Bertelli, M. (2020). Bacteriophages presence in nature and their role in the natural selection of bacterial populations. *Acta Bio Medica: Atenei Parmensis*, *91*(Suppl 13). **DOI: 10.23750/abm.v91i13-S.10819**

Negrut, N.; Khan, S. A.; Bungau, S.; Zaha, D. C.; Anca, C. A.; Bratu, O., and Ionita-Radu, F. (2020). Diagnostic challenges in gastrointestinal infections. *Rom J Mil Med*, *123*, 83-90. **DOI: 10.55453/rjmm.2020.123.2.1**

Thung, T. Y.; Siti Norshafawatie, B. M. F.; Premarathne, J. M. K. J. K.; Chang, W. S.; Loo, Y. Y.; Kuan, C. H.; New, C. Y.; Ubong, A.; Ramzi, O. S. B.; Mahyudin, N. A. Dayang, F. B.; Che Wan Jasimah, W. M. R. and Son, R. (2017). Isolation of food-borne pathogen bacteriophages from retail food and environmental sewage. *International Food Research Journal*, *24*(1), 450-454.

Pawar, M. G. M. and Salgaonkar, B. B. (2023). The Biodiversity and Ecology of the Fragile Ecosystem of Goa: The Mangroves and the Saltpans.

Ranveer, S. A.; Dasriya, V.; Ahmad, M. F.; Dhillon, H. S.; Samtiya, M.; Shama, E.

and Puniya, A. K. (2024). Positive and negative aspects of bacteriophages and their immense role in the food chain. *npj Science of Food*, *8*(1), 1. **DOI:10.1038/s41538-023-00245-8**

Sanz-Gaitero, M.; Seoane-Blanco, M. and van Raaij, M. J. (2021). Structure and function of bacteriophages. *Bacteriophages: Biology, Technology, Therapy*, 19-91.

Simmons, E. L.; Bond, M. C.; Koskella, B.; Drescher, K.; Bucci, V. and Nadell, C. D. (2020). Biofilm structure promotes coexistence of phage-resistant and phage-susceptible bacteria. *MSystems*, *5*(3), 10-1128. **DOI: 10.1128/msystems.00877-19.**

Sliwka, P.; Ochocka, M. and Skaradzińska, A. (2022). Applications of bacteriophages against intracellular bacteria. *Critical Reviews in Microbiology*, *48*(2), 222-239. **DOI: 10.1080/1040841X.2021.1960481**

Tan, C. W.; Rukayadi, Y.; Hasan, H.; Abdul-Mutalib, N. A.; Jambari, N. N.; Hara, H. and Radu, S. (2021). Isolation and characterization of six *Vibrio parahaemolyticus* lytic bacteriophages from seafood samples. *Frontiers in Microbiology*, 12, 616548. **DOI: 10.3389/fmicb.2021.616548**

Wdowiak, M.; Paczesny, J. and Raza, S. (2022). Enhancing the stability of bacteriophages using physical, chemical, and nano-based approaches: A review. *Pharmaceutics*, *14*(9), 1936. **DOI:10.3390/pharmaceutics14091936**

Węgrzyn, G. (2022). Should bacteriophages be classified as parasites or predators?. *Polish Journal of Microbiology*, *71*(1), 3-9. **DOI: 10.33073/pjm-2022-005**