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Original Research Articles

Application of Bacteriophages to Treat Toxic Gas-producing Bacteria *Desulfovibrio* spp. in Shrimp Ponds

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Desulfovibrio spp. bacteria pose significant challenges in shrimp aquaculture by producing hydrogen sulfide (H_2S), which depletes oxygen levels and hinders shrimp growth. The increasing prevalence of antibiotic-resistant bacteria necessitates the exploration of alternative control methods. This study investigates the potential of bacteriophages as a biological solution to inhibit *Desulfovibrio* spp. in shrimp ponds. Six bacteriophage strains (ST231, ST22, φMix 1, φS8, φS9, and φMix 2) were evaluated using plaque assays at three time points (3, 6, and 18 hours). Results demonstrated that most bacteriophages significantly reduced bacterial density over time, with ST22 showing the most substantial inhibition ($p<0.05$). Genomic analysis of bacteriophage ST22, published in the GenBank database, revealed genes related to *Desulfovibrio* spp., specifically Deltaproteobacteria and Desulfobacteraceae. The study also found that bacteriophage treatment led to decreased H_2S and NH_3 levels and increased dissolved oxygen in simulated pond environments. Morphological changes in bacterial colonies post-treatment further supported the phages' inhibitory effects. These findings suggest that bacteriophage ST22 is a promising biological alternative to antibiotics for preventing and controlling diseases caused by *Desulfovibrio vulgaris* in shrimp aquaculture, though further research is needed to assess long-term ecological impacts and efficacy in complex pond ecosystems.

INTRODUCTION

Vietnam's favorable conditions support significant aquaculture development, particularly shrimp farming. White leg shrimp (*Litopenaeus vannamei*) and black tiger shrimp (*Penaeus monodon*) are major aquatic export products. However, *Desulfovibrio* spp., H_2S-producing bacteria, poses a significant threat to this industry. These sulfate-reducing bacteria thrive in anaerobic environments, converting sulfate to hydrogen sulfide.[1] The resulting H_2S, a metabolic byproduct, is harmful at high concentrations. It is carcinogenic, toxic to intestinal cells, inhibits oxygen binding to cytochrome c, disrupts oxidative phosphorylation and ATP formation, and can mutate DNA and disrupt protein structures. The increasing incidence of bowel diseases in Western countries highlights the importance of research on managing *Desulfovibrio* spp. and its impact on gut health.[2] In shrimp ponds, *Desulfovibrio* spp. proliferate in high-organic-matter environments, such as those with bottom residues, sludge, and untreated crop liners. This leads to toxic gas accumulation, particularly H_2S, under the pond bottoms.[2] Waste and leftover feed contribute to anaerobic conditions, resulting in foul odors and potential shrimp mortality. Effective pond management is crucial to prevent organic pollution and maintain shrimp quality, as neglect leads to substantial farmer losses.[3]

Antibiotic use in aquaculture results in antibiotic residues in water and sludge, promoting antibiotic-resistant bacteria. Bacteriophages, viruses that infect bacteria, offer a potential alternative for controlling bacterial diseases. Discovered by William Twort in 1915 and Felix d'Herelle in 1917,[4] bacteriophages are a promising biocontrol method in aquaculture, minimizing environmental and human impacts. This approach, initially used in Japan,[5] has gained significant scientific interest. Previous studies have demonstrated the lytic activity of bacteriophages against various *Desulfovibrio* species.[6-10] This study aimed to identify and evaluate potential bacteriophage strains for inhibiting *Desulfovibrio* spp. in shrimp ponds.

MATERIALS AND METHODS

MATERIALS

Desulfovibrio sp. bacteria were obtained from the Can Tho University project: "Application of bacteriophages to treat

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toxic gas-producing bacteria causing decreased oxygen levels in shrimp ponds" (Project code: TSV2021-156). The genus-level identification, based on morphological, physiological, and biochemical characteristics, had been reported by Van & Thu.[11] This study further identifies the species level using 16S rRNA gene sequencing. Bacteriophage strains ST231, ST22, φMix 1 (M1: ST231 + ST22), φS8, φS9, and φMix 2 (M2: S8 + S9) (isolated in Tra Vinh province) which were provided by the Molecular Biology Laboratory, Institute of Biotechnology and Food Technology, Can Tho University.

ISOLATION AND IDENTIFICATION OF *DESULFOVIBRIO* SPP. BACTERIA BY 16S RRNA GENE SEQUENCING

Bacterial DNA was extracted, and PCR was performed using 16S rRNA primers. PCR products were electrophoresed on agarose gel to confirm the expected ~1500 bp band. Samples with clear bands were sent to the 1st BASE laboratory for sequencing. The 16S rRNA gene sequences were compared using the BLAST program in the NCBI database to identify the bacterial strains.

INVESTIGATION OF THE INHIBITORY EFFECTS OF BACTERIOPHAGE ON *DESULFOVIBRIO* SPP. BACTERIA IN THE LABORATORY AND 2-LITER JAR MODELS

Seven liters of shrimp pond water were divided into seven 1-liter plastic jars. Water parameters (dissolved oxygen (DO), H_2S, and NH_3/NH_4^+ levels) were monitored. Pre-inoculation agar plating was performed. Each jar received 2 mL of the *Desulfovibrio vulgaris* strain 12Đ (species identified through 16S rRNA sequencing), 7 mL of TSB medium, and 50 mL bottom sludge. Then, 250 µL of each bacteriophage (or 125 µL of each phage for φMix) was inoculated. Post-inoculation agar plating was performed at 3, 6, and 18 hours at a 10^{-4} dilution. Water parameters were monitored at 3 and 18 hours. Dissolved oxygen (DO), H_2S, and NH_3/NH_4^+ levels were measured using Sera test kits.

EVALUATION OF THE EFFECTS OF BACTERIOPHAGES ON COLONY MORPHOLOGY OF *DESULFOVIBRIO* SPP.

Using a plaque assay method, 200 µL of control (shrimp pond water inoculated with strain 12Đ) and 200 µL from jars inoculated with bacteriophages were serially diluted to 10^{-4} and spread onto TCBS agar plates supplemented with 1.5% agar. Plates were incubated at 32°C for 24 hours, and colony morphology and CFU were assessed at 3, 6, and 18 hours post-bacteriophage inoculation.

PHAGE GENOME SEQUENCING AND BIOINFORMATICS ANALYSIS

DNA extraction was performed using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. DNA quality and quantity were assessed using a NanoDrop spectrophotometer. Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and sequenced on an Illumina NextSeq 500 platform (2 x 150 bp paired-end). Raw reads were quality-controlled using FastQC v0.11.9 and de novo assembled using Unicycler (v0.4.8). All predicted open reading frames (ORFs) were compared against the NCBI non-redundant protein database using BLASTp. Whole genome alignments were performed using progressiveMauve. The genome maps were visualized using CGView.[12]

STATISTICAL ANALYSIS

ANOVA (Tukey's and Fisher's tests) was performed using MS Excel and Minitab 16. Data are presented as means ± standard deviations (SDs) of triplicate experiments.

RESULTS

IDENTIFICATION OF H_2S-PRODUCING BACTERIA

PCR electrophoresis results of the 16S rRNA gene segment showed that six samples had bands at approximately 1500 bp, consistent with the expected size. 16S rRNA gene sequencing was performed to identify the H_2S-producing bacterial strain 12Đ. Phylogenetic analysis based on the 16S rRNA gene sequence placed strain 12Đ within the *Desulfovibrio* genus, showing close relatedness to *D. vulgaris* (**Figure 1**). Although the 16S rRNA gene sequence of strain 12Đ has not yet been submitted to GenBank, the phylogenetic analysis strongly suggests it belongs to the *D. vulgaris* species.

GAS PRODUCTION IN SHRIMP FARMING WATER BEFORE AND AFTER INOCULATION WITH *D. VULGARIS*

Table 1 shows that before bacterial inoculation (pH=9, salinity 15.6‰), natural shrimp pond water had negligible NH_3 (0 mg/L) and H_2S (0.002 mg/L), and a moderate NO_3^- concentration (50 mg/L). Dissolved O_2 was 4 mg/L). After inoculation with *D. vulgaris* strain 12Đ, pH decreased (pH=8), but salinity remained at 15.6‰. H_2S increased to 0.97 mg/L, NH_3 increased to 5 mg/L (a dangerous level for shrimp farming), and DO decreased to 6 mg/L. NO_3^- levels decreased significantly.

It can be predicted that the beneficial bacteria had died off due to the significant increase of harmful bacteria in the culture jar (**Figure 2A**). In addition, pre-inoculation agar plating results showed that the bacterial concentration in the jar was still low, and the gases produced were not yet at a level that killed the cultured shrimp.

However, post-inoculation agar plating results showed that the concentration of *D. vulgaris* increased significantly, as evidenced by the black colonies on the TCBS medium (**Figure 2B**). This is explained by the fact that *D. vulgaris* bacteria produce H_2S gas, which reacts with Fe^{3+} in TCBS medium to form ferric sulfide (FeS^- black precipitate), indicating the presence of black on the colonies.[2]

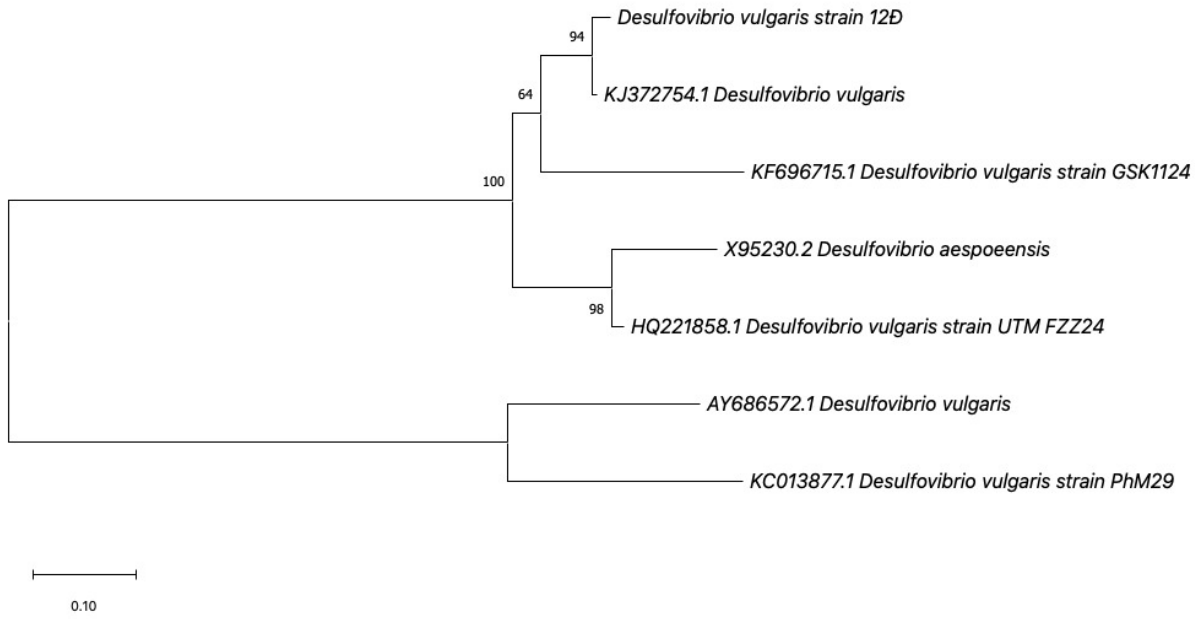

Figure 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain 12Đ to other *Desulfovibrio* species.

Table 1. Summary of NH_3, NO_3^-, H_2S, pH, O_2 parameters tested using Sera test kits

	pH	Salinity	NH_3	H_2S	NO_3^-	O_2
Before inoculation (natural shrimp pond water)	9	15.6‰	0 mg/L	0.002 mg/L	50 mg/L	4 mg/L
After 12Đ inoculation	8	15.6‰	5 mg/L	0.97 mg/L	0 mg/L	6 mg/L

GAS PRODUCTION AFTER BACTERIOPHAGE INOCULATION

Table 2 shows the results of gas production at 3- and 18-hours post-bacteriophage inoculation. In the control, H_2S and NH_3 levels remained high at both time points. At 18 hours, bacteriophage treatments, particularly ST22, φM1, φS9, φM2 significantly decreased H_2S to levels safe for for shrimp (≤0.05 mg/L). NH_3 levels were also significantly lower. In treatments with ST22 and φM1, DO increased to 8 mg/L, indicating effective inhibition of H_2S production.

INHIBITORY EFFECTS OF BACTERIOPHAGE ON *D. VULGARIS*

Investigating the colony morphology of *D. vulgaris* after bacteriophage inoculation revealed that almost all colonies lost their black color and turned blue at 3, 6, and 18 hours (Figure 3). Almost all colonies lost their black color, indicating that the bacteriophages may have inhibited the H_2S-producing ability of bacteria. Specifically, bacteriophages infected and inhibited specific bacterial strain(s), thus inhibiting bacterial virulence genes, causing bacteria to lose their ability to produce toxic gases.

Three hours and 6 hours show that the colonies gradually lost their back color at the edges, indicating that the φM1 and φM2 bacteriophages were able to eliminate the H_2S-producing ability of bacteria or had superior properties when combining both phages in one treatment. At 18 hours, colonies in Figures 3C and **3G** show that after the bacteriophages inhibited the gas-producing ability of bacteria, the colonies still had black centers. They did not completely inhibit *D. vulgaris* bacteria. However, in Figures 3D and **3F**, the colonies almost completely lost their black color. The specificity of bacteriophages can explain this, so the inhibitory abilities differed among treatments.

The colony counts assessment results at 3, 6, and 18 hours showed that all bacteriophages can reduce colony counts over time (Table 3). Phage strain ST22, which reduced the colony counts to 6.53 log(CFU/mL) compared to the control, showed the highest inhibitory efficiency at 3 hours, similar to phage strain S8, which reduced the colony density to 6.44 log(CFU/mL) compared to the control evidence of the highest inhibitory efficiency against *D. vulgaris* bacteria at 6 hours.

At 18 hours, phage strain ST22 showed the highest efficiency in reducing colony density, 6.46 log(CFU/mL) compared to the control, and the remaining treatments also

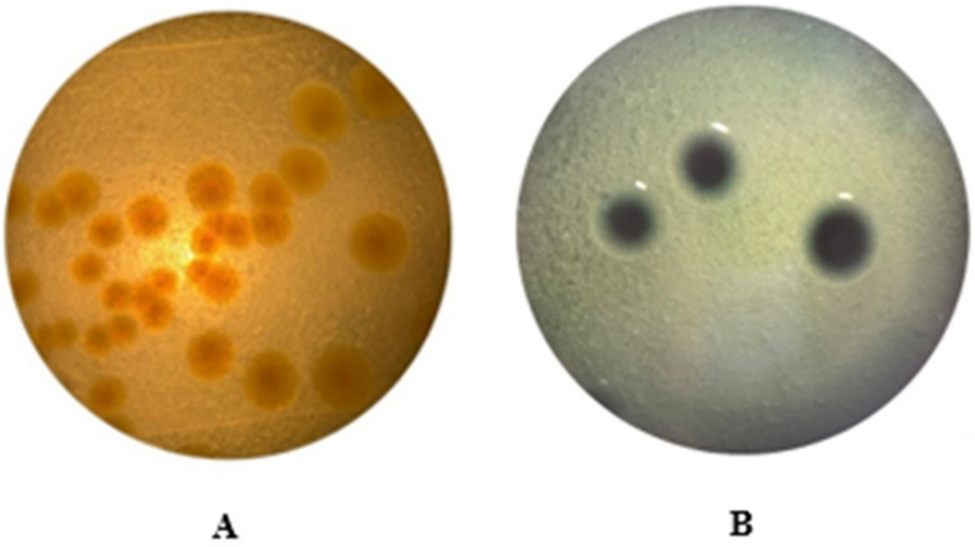

Figure 2. Pre-inoculation (A) and post-inoculation (B) agar plating results of pond bottom sludge water on TCBS medium.

Table 2. Summary of monitored parameters after bacteriophage inoculation at three hours and 18 hours

Parameters		pH	H_2S (mg/L)	NH_3 (mg/L)	O_2 (mg/L)	Temperature (°C)	Salinity (‰)
Control (12Đ)	At 3 hours	8	1.45	0.27	0.5	30.2	15.5
	At 18 hours	9	1.45	1.8	2	30.2	15.5
ST231	At 3 hours	8	0.97	0.11	0.5	29.8	15.3
	At 18 hours	8	0.05	0.11	0.5	29.8	15.3
ST22	At 3 hours	8	0.97	0.11	0.5	30.2	15.4
	At 18 hours	8	0.025	0.05	8	30.2	15.4
φM1	At 3 hours	8	0.97	0.11	0.5	30.2	15.5
	At 18 hours	8	0.025	0.27	8	30.2	15.5
φS8	At 3 hours	8	0.97	0.11	0.5	30.2	15.5
	At 18 hours	8	0.05	0.05	0.5	30.2	15.5
φS9	At 3 hours	8	0.97	0.11	0.5	30.2	15.5
	At 18 hours	7	0.025	0.006	2	30.2	15.5
φM2	At 3 hours	8	0.97	0.11	0.5	30.2	15.5
	At 18 hours	8	0.025	0.11	2	30.2	15.5

reduced colony density. Thus, it can be seen that these bacteriophage strains reduced colony counts over time.

GENOME ANALYSIS OF BACTERIOPHAGES ST231 AND ST22 CAPABLE OF INHIBITING *D. VULGARIS*

Phage ST231 and ST22 have complete genomes consisting of circular DNA molecules. The annotation information for ST231 and ST22 can be found on GenBank with accession numbers OP921041.1 and OQ957557.1, respectively. The genomes are visualized in **Table 4**. Bacteriophage ST231 has a genome size of 41,280 bp with a GC percentage of 53.9% and encodes 61 ORFs. Bacteriophage ST22 has a genome size of 45,779 bp with a GC percentage of 54.7% and encodes 62 ORFs. Both phages belong to the Caudoviricetes class. The genomes do not contain tRNA genes, lysogenic phage-related markers, toxin genes, virulence genes, or antibiotic resistance genes, making them potentially safe agents for controlling *D. vulgaris* infections in shrimp ponds.

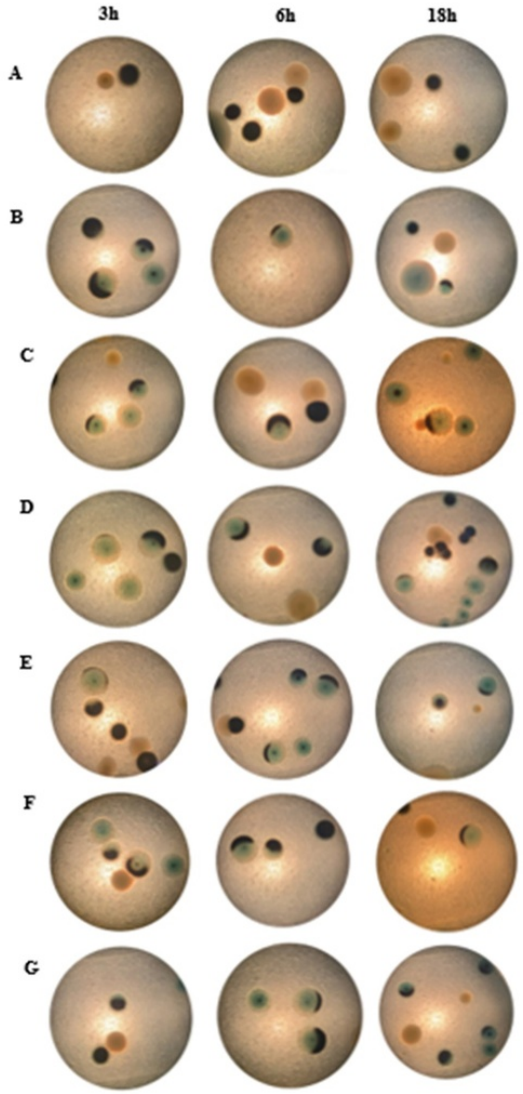

Figure 3. The colony morphology of *D. vulgaris* after bacteriophage inoculation at three hours, six hours and 18 hours.

A – control, B – 12Đ+ST231, C – 12Đ+ST22, D – 12Đ+φM1, E – 12Đ+φS8, F – 12Đ+φS9, G – 12Đ+φM2.

DISCUSSION

This study demonstrates the potential of bacteriophages as biological control agents against *D. vulgaris* in shrimp aquaculture. The reduction in H_2S and NH_3 levels, coupled with increased dissolved oxygen, supports previous findings on phage treatment of sulfate-reducing bacteria in wastewater.[13] The loss of black coloration in *D. vulgaris* colonies suggests inhibition of H_2S production, possibly due to phage interaction with bacterial virulence genes.[14]

The bacteriophages ST22 demonstrated the most promising results in the tests. Importantly, no virulence factors or antibiotic resistance genes were found in either phage genome, indicating their potential for safe use in biocontrol. Key structural genes (major capsid protein, tail protein, portal protein), lysis-related genes (endolysins and holins), and several DNA replication and metabolism genes were identified. The genome sizes and G+C content of ST231 and ST22 are typical for tailed phages. Their modular genome organization, including structural genes, lysis modules, and DNA replication genes, is consistent with their lytic lifestyle, which is advantageous for phage therapy applications.[15,16] The identification of lysis-related genes, particularly endolysins, in both phages, is significant for their potential use in biocontrol.[17] This host-phage relationship is crucial for effective phage therapy.[18]

The gradual reduction in bacterial counts suggests a sustained effect of phage treatment,[19] while the enhanced efficacy of phage cocktails (φM1 and φM2 in some cases) aligns with previous research.[20] However, laboratory conditions may not fully reflect complex shrimp pond ecosystems. Factors such as environmental fluctuations, other microorganisms, and potential phage resistance development must be considered for field applications.[21] The genomic similarities of ST231 to *Vibrio* phages and ST22 to *Enterobacter* phages suggest the potential for controlling multiple bacterial species in shrimp ponds,[22] which broadens the potential application for these phages beyond *D. vulgaris* control. The long-term ecological impacts of phage introduction require further investigation.[23]

Future research should focus on optimizing phage dosage and timing of application, as well as investigating potential synergistic effects with other biocontrol agents. Moreover, studies on the potential development of phage resistance in *D. vulgaris* and strategies to mitigate this risk would be valuable for sustainable long-term application of phage therapy in aquaculture.

CONCLUSIONS

This study demonstrates the potential of bacteriophages as an effective biological control agent against *D. vulgaris* in shrimp aquaculture. Through 16S rRNA sequencing, we

Table 3. The colony counts of *D. vulgaris* after bacteriophage inoculation (log(CFU/mL))

	Control	ST231	ST22	φM1	φS8	φS9	φM2
At 3 hours	6.94 ± 0.01^{a}	6.57 ± 0.05^{c}	6.53 ± 0.07^{c}	6.67 ± 0.09^{bc}	6.64 ± 0.13^{bc}	6.83 ± 0.02^{ab}	6.83 ± 0.02^{ab}
At 6 hours	7.17 ± 0.07^{a}	6.61 ± 0.1^{bc}	6.49 ± 0.1^{c}	6.57 ± 0.04^{bc}	6.44 ± 0.05^{c}	6.78 ± 0.05^{b}	6.59 ± 0.12^{bc}
At18 hours	7.24 ± 0.07^{a}	6.67 ± 0.02^{b}	6.46 ± 0.06^{b}	6.71 ± 0.17^{b}	6.64 ± 0.06^{b}	6.60 ± 0.05^{b}	6.68 ± 0.14^{b}

Table 4. Identified genes in bacteriophages ST22 and ST231 and their predicted functions

ORF	Accession Number	Predicted function	Accession Number	Predicted function
		Phage ST22		Phage ST231
ORF1	WKV24410.1	terminase	WAV88248.1	hypothetical protein
ORF2	WKV24411.1	hypothetical protein	WAV88249.1	hypothetical protein
ORF3	WKV24412.1	hypothetical protein	WAV88250.1	hypothetical protein
ORF4	WKV24413.1	hypothetical protein	WAV88251.1	hypothetical protein
ORF5	WKV24414.1	hypothetical protein	WAV88252.1	hypothetical protein
ORF6	WKV24415.1	hypothetical protein	WAV88253.1	hypothetical protein
ORF7	WKV24416.1	hypothetical protein	WAV88254.1	hypothetical protein
ORF8	WKV24417.1	hypothetical protein	WAV88255.1	hypothetical protein
ORF9	WKV24418.1	hypothetical protein	WAV88256.1	hypothetical protein
ORF10	WKV24419.1	DNA primase/polymerase	WAV88257.1	hypothetical protein
ORF11	WKV24420.1	hypothetical protein	WAV88258.1	hypothetical protein
ORF12	WKV24421.1	Sak4-like ssDNA annealing protein	WAV88259.1	hypothetical protein
ORF13	WKV24422.1	hypothetical protein	WAV88260.1	hypothetical protein
ORF14	WKV24423.1	hypothetical protein	WAV88261.1	hypothetical protein
ORF15	WKV24424.1	hypothetical protein	WAV88262.1	terminase large subunit
ORF16	WKV24425.1	putative CRISPR-associated exonuclease	WAV88263.1	Portal protein
ORF17	WKV24426.1	ATP-dependent DNA helicase	WAV88264.1	hypothetical protein
ORF18	WKV24427.1	DNA nuclease	WAV88265.1	hypothetical protein
ORF19	WKV24428.1	DNA polymerase III beta subunit	WAV88266.1	hypothetical protein
ORF20	WKV24429.1	HNH endonuclease	WAV88267.1	hypothetical protein
ORF21	WKV24430.1	hypothetical protein	WAV88268.1	hypothetical protein
ORF22	WKV24431.1	DNA N-6-adenine-methyltransferase	WAV88269.1	hypothetical protein
ORF23	WKV24432.1	hypothetical protein	WAV88270.1	hypothetical protein
ORF24	WKV24433.1	DNA stabilization protein	WAV88271.1	DNA stabilization protein
ORF25	WKV24434.1	hypothetical protein	WAV88272.1	hypothetical protein
ORF26	WKV24435.1	DNA stabilization protein/tail needle	WAV88273.1	DNA stabilization protein/tail needle
ORF27	WKV24436.1	hypothetical protein	WAV88274.1	hypothetical protein
ORF28	WKV24437.1	DNA transfer protein	WAV88275.1	DNA transfer protein
ORF29	WKV24438.1	Putative DNA transfer protein	WAV88276.1	Putative DNA transfer protein
ORF30	WKV24439.1	Transglycosylase	WAV88277.1	Transglycosylase
ORF31	WKV24440.1	Colanic acid biosynthesis protein wcaM	WAV88278.1	Colanic acid biosynthesis protein
ORF32	WKV24441.1	hypothetical protein	WAV88279.1	hypothetical protein
ORF33	WKV24442.1	hypothetical protein	WAV88280.1	hypothetical protein
ORF34	WKV24443.1	C-5 cytosine specific DNA methylase	WAV88281.1	Single-stranded DNA-binding protein
ORF35	WKV24444.1	Putative recombinase	WAV88282.1	hypothetical protein
ORF36	WKV24445.1	PD-(D/E)XK nuclease-like domain-containing protein	WAV88283.1	PD-(D/E)XK nuclease-like domain-containing protein
ORF37	WKV24446.1	type III restriction endonuclease	WAV88284.1	type III restriction endonuclease
ORF38	WKV24447.1	DEAD/DEAH box helicase family protein	WAV88285.1	DEAD/DEAH box helicase family protein
ORF39	WKV24448.1	putative DNA primase	WAV88286.1	putative DNA primase

ORF	Accession Number	Predicted function	Accession Number	Predicted function
ORF40	WKV24449.1	minor tail protein	WAV88287.1	hypothetical protein
ORF41	WKV24450.1	hypothetical protein	WAV88288.1	putative DNA methylase
ORF42	WKV24451.1	putative minor tail protein	WAV88289.1	hypothetical protein
ORF43	WKV24452.1	tail tape measure protein	WAV88290.1	hypothetical protein
ORF44	WKV24453.1	DUF1799 domain-containing protein	WAV88291.1	putative DNA cytosine C5 methyltransferase
ORF45	WKV24454.1	tail assembly chaperone	WAV88292.1	hypothetical protein
ORF46	WKV24455.1	major tail protein	WAV88293.1	hypothetical protein
ORF47	WKV24456.1	DUF4128 domain-containing protein	WAV88294.1	hypothetical protein
ORF48	WKV24457.1	hypothetical protein	WAV88295.1	hypothetical protein
ORF49	WKV24458.1	major tail subunit	WAV88296.1	major tail subunit
ORF50	WKV24459.1	tail assembly-like protein	WAV88297.1	tail assembly-like protein
ORF51	WKV24460.1	endolysin	WAV88298.1	endolysin
ORF52	WKV24461.1	holin family protein	WAV88299.1	holin family protein
ORF53	WKV24462.1	hypothetical protein	WAV88300.1	hypothetical protein
ORF54	WKV24463.1	hypothetical protein	WAV88301.1	hypothetical protein
ORF55	WKV24464.1	hypothetical protein	WAV88302.1	hypothetical protein
ORF56	WKV24465.1	hypothetical protein	WAV88303.1	hypothetical protein
ORF57	WKV24466.1	hypothetical protein	WAV88304.1	hypothetical protein
ORF58	WKV24467.1	hypothetical protein	WAV88305.1	hypothetical protein
ORF59	WKV24468.1	hypothetical protein	WAV88306.1	hypothetical protein
ORF60	WKV24469.1	immunoglobulin domain-containing protein	WAV88307.1	hypothetical protein
ORF61	WKV24470.1	head morphogenesis	WAV88308.1	hypothetical protein
ORF62	WKV24471.1	DUF4055 domain-containing protein	N/A	N/A

successfully identified *D. vulgaris* in shrimp pond environments. Bacteriophage treatment resulted in significant reductions of H_2S and NH_3 levels, coupled with increased dissolved oxygen, indicating the phages' ability to inhibit the gas-producing capabilities of *D. vulgaris*. Morphological changes in bacterial colonies post-treatment further supported this inhibition. Among the tested phages, ST22 showed the most promising results in reducing bacterial colony counts. Genomic analysis of bacteriophages ST231 and ST22 showed that they are classified under the *Caudoviricetes* class. The genomes do not possess tRNA genes, lysogenic phage-related markers, toxin genes, virulence genes, or antibiotic resistance genes. This suggests that these phages could be safe options for managing *D. vulgaris* infections in shrimp ponds. The gradual reduction in bacterial counts over time with all tested phages suggests a sustained effect, while combinations of phages showed enhanced efficacy in some cases. These results indicate that bacteriophages, particularly strain ST22, offer a promising biological alternative to antibiotics for preventing and controlling diseases caused by *D. vulgaris* in shrimp aquaculture. However, further research is needed to evaluate the long-term ecological impacts and efficacy of phage treatment in complex shrimp pond ecosystems before large-scale application can be recommended.

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AUTHORS' CONTRIBUTION

Conceptualization: Truong Thi Bich Van; Methodology: Truong Thi Bich Van, Tran Vo Minh Thu, Van-Thanh Vo; Formal analysis and investigation: Truong Thi Bich Van, Tran Vo Minh Thu, Van-Thanh Vo, Nguyen Thi Loan Anh; Writing - original draft preparation: Tran Vo Minh Thu, Van-Thanh Vo, Nguyen Thi Loan Anh; Writing - review and editing: All authors; Supervision: Truong Thi Bich Van

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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