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Abundance and ecological footprint of *Pseudoalteromonas* phage vB_PhoS_XC in the *Ulva prolifera* green tide

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Pseudoalteromonas is a ubiquitous and abundant genus of marine bacteria commonly associated with algae. In this study, a novel siphoviral-morphological bacteriophage, vB_PhoS_XC, was isolated from the coastal seawaters of Qingdao (China) during a bloom of the *Ulva prolifera* (*U. prolifera*) green tide. The morphology of this phage (icosahedron head 51 ± 1 nm in diameter; a tail length of 86 ± 1 nm) was characterized through transmission electron microscope. The biological properties of this virus showed a short latent period (45 minutes), a large burst size (241 virions per cell) and a relatively wide range of temperatures/pH level tolerance (-20°C to 45°C and pH 4 to pH 10, respectively). The vB_PhoS_XC has a 46,490-bp double-stranded DNA genome with a G+C content of 40.0%, and encodes 72 open reading frames (ORFs). Thirty-five of these ORFs were assigned into known functions based on BLAST-based algorithm against NR database of GenBank. In addition, eco-genomic analysis provides the evidence of vB_PhoS_XC accompanied by bloom of *U. prolifera*, and confirmed the high expression of two phosphatase-metabolism-related auxiliary metabolic genes (AMGs). This study provides new insights into the functional and ecological roles of the *Pseudoalteromonas* phage vB_PhoS_XC, shedding light on the virological study approach combined with traditional isolation and meta-omics data.

KEYWORDS

bacteriophage, *Pseudoalteromonas*, *Ulva prolifera* blooms, genomic and meta-omics analysis, ecological footprint

Introduction

In coastal marine ecosystems, algae are a naturally abundant food source for marine organisms and contribute to the production of polysaccharide-rich detritus (Jain et al., 2020; Akter et al., 2022). However, macroalgae blooms create great pressure on these ecosystems, resulting in hypoxia and acidification (Zhang et al., 2019). The largest causal agent of green tides is the macroalgae *Ulva prolifera* (*U. prolifera*), which has been affecting Qingdao since 2007. Following a bloom, huge amounts of dissolved organic matter (DOM) are produced due to the degradation of *U. prolifera* (Zhang and Wang, 2017). This leads to serious negative economic impacts and complex ecological consequences, most notably on the structure of the microbial community (Xiao et al., 2021; Zhang et al., 2021). Nevertheless, the majority of studies on *U. prolifera* control and microenvironment typically concentrated on the relationships between *U. prolifera* and bacteria, for instance, changes in bacterial communities and responses to *U. prolifera* without taking into consideration the potential contribution of viruses (Qu et al., 2020; Liang et al., 2021; Qu et al., 2021; Zhao et al., 2022).

Viruses are the most abundant and diverse biological entities in the ocean (Culley et al., 2006), playing an important role in global biogeochemical cycles (Fuhrman, 1999). They are also the most abundant and genetically diverse “life forms” on Earth (Fuhrman, 1999). Viruses mediate horizontal gene transfer by infecting host cells, and they also play a key role in the formation of bacterial community structure. Moreover, they can reduce the abundance of the major host by infecting and inducing the community structure into homeostasis (Zhang et al., 2007). The largest community of viruses in marine ecosystems are bacteriophages, which are bacterial specific. It is estimated that abundance of bacteriophages is around 10–15 times higher than bacteria (Culley et al., 2006), and the expression of phage-encoded auxiliary metabolic genes (AMGs) can influence bacterial host with reprogramming metabolic progress and improves their adaptability to environmental changes (Gregory et al., 2019; Zimmerman et al., 2020).

Pseudoalteromonas is the most abundant bacteria genus found in coastal seawater and is dominant during *U. prolifera* blooms (Wang et al., 2020; Qu et al., 2021). Metabolically, this species exhibits high productivity and can effectively use a variety of carbon sources, providing a competitive advantage over other microorganisms. Further, it has been explored to secrete a range of extracellular substances, including enzymes, toxins, antibiotics and polysaccharides (Ivanova et al., 2003), and found to make significant contributions to dissolved algae through the action of alginate lyases. This organism’s strong capacity to produce extracellular degrading enzymes, as well as a variety of bioactive substances, leads to its important role in marine biogeochemical cycles (Bowman, 2007). Although *Pseudoalteromonas* is a significant alginate-degrading group in the ocean, with its presence being particularly prominent in coastal seawater (Rong et al., 2018; Ren et al., 2022), few *Pseudoalteromonas* phages have been isolated. Thus, we sought to explore the possible regulatory role of viruses in the *U. prolifera* blooms in the coastal marine ecosystems of Qingdao in this study.

To do this, the *Pseudoalteromonas* phage vB_PhoS_XC was isolated from these waters, its biological characteristics were investigated, and its genome was analyzed. Additionally, the metagenome and metatranscriptome outcomes of the phage-host interaction were used as models to suggest that the virus could influence the dynamic population of *U. prolifera*. This work thus adds to our understanding of *U. prolifera* blooms and provides a framework for further studies on the complex interactions between *U. prolifera*, *Pseudoalteromonas*, and their phages.

Materials and methods

Sampling and virion enrichment

Seven samples were collected from the coastal seawaters of Qingdao between July to December in 2020 year. Eighty-liters of surface seawater were sampled from the coast of Qingdao (36.06° N, 120.31° E), and then pre-filtered on nylon sieves with a mesh of 200 μm and 20 μm to remove large zooplankton. Seawater samples were pre-filtered 3 μm filters and then followed by filtration through 0.22 μm Polycarbonate Membranes filters (Millipore, MA, USA) using a peristaltic pump (Stewart et al., 2012; Pesant et al., 2015). Filters were transferred immediately to DNase/RNase-Free tubes with RNAlater and stored at -80°C for RNA extraction. To further concentrate the filtered seawater containing viruses, tangential flow filtration (laboratory scale, 50 kDa; Millipore) was used to reduce samples to 100 mL for DNA extraction. The samples and the original seawater were stored at 4°C in the dark until experimentation (Wang et al., 2015).

Preparation of bacterial strain

Host bacterial strain was isolated from the original seawater at the coast seawaters of Qingdao during the *U. prolifera* blooms in 2020 year, and then incubated in liquid Zobell medium at 28°C for next experiments (Liu et al., 2018). The 16S rRNA gene of host was amplified by PCR. The phylogenetic tree was calculated on the basis of the 16S rRNA sequences using IQ-TREE with MFP model as the best-fitted substitution model undergoing 1,000 bootstraps (Supplementary Figure S1).

Isolation, purification of bacteriophage

The phage vB_PhoS_XC was prepared using the standard virus enrichment approach and doubled-layer agar methods (Jamaludeen et al., 2007), followed by using the soft-agar overlay method for plaque analysis (Hyman and Abedon, 2010). Briefly, 1 mL host cell culture was incubated with 0.2 mL seawater, and then was filtered through 0.22 μm pore-size membranes (Millipore) for 20 min. The mixture was placed into 5 mL of soft and warm agar (0.6%), and poured onto petri dishes to form plaques. Phages were purified by picking a single plaque, suspending it in SM buffer (100 mM NaCl, 81.2 mM MgSO_4 , 50 mM Tris HCl [pH 7.5], 0.01%

gelatin) for 1 h at 37°C. The suspension was filtered through 0.22 µm pore-size membranes (Millipore), and was used to obtain the plaque through double-layer plate method. The purification step was repeated for three times. Then the purified phages were amplified and stored at 4°C.

Morphological study by transmission electron microscopy

20 µL viral suspension was negatively stained with 2% uranyl acetate, and was examined by energy-filtering transmission electron microscopy (TEM, JEOL Model JEM-1200EX) at 100 kV at NICEM to obtain the viral morphological data (Li Y et al., 2016). The average size of virion was determined by measuring three phage particles within the same field of vision.

One-step growth assay

The one-step growth curve assay was used to identify the burst size of the phage vB_PhoS_XC following the previous method with some modifications (Middelboe et al., 2010; Ma et al., 2021). The burst size was calculated by dividing the final number of virions by the initial number of infected host cells at the beginning of assay (Wang et al., 2017). A bacterial culture in the exponential growth phase (2×10^8 CFU/mL) was mixed with 1 mL of the phage suspension (MOI=0.01) at 25°C for 20 min. After centrifugation at 12,800 g for 30 seconds to remove unabsorbed phage, samples were re-suspended to 50 mL liquid Zobell medium. The re-suspended virions were used to infect bacteria with the 5-min/10-min/30-min interval in first/second/third hour, respectively (Gong et al., 2017). This assay was re-performed by three times (Li et al., 2016). The virion/bacteria co-culture was plated on a double-layer plate to incubate overnight. The number of phage plaques were counted to determine the titers of the phage at different time points to assess its growth states.

pH/thermal stability

We tested pH/thermal stability to gain the information on the biological properties of the phage vB_PhoS_XC. Briefly, to test capsid tolerance to pH, 100 µL phage suspension mixed with 900 µL SM buffer with a range of pH from 3 to 12 at 30 °C for 2 h. To test the capsid tolerance to temperature, the phage suspension was incubated at -20°C, 4°C, 25°C, 35°C, 45°C, 55°C, 65°C, and 75°C for 2 h, then incubated with host at 25°C for 20min. The double-layer plate method was used to calculate the loss of virus titer of each sample. Each sample was performed in triplicate.

Genome sequencing and function analysis

Bacteriophage DNA was extracted by Virus DNA Kit (OMEGA) and sequenced using the Illumina HiSeq PE150

paired-end sequence method by Novogene (Tianjin, China) company (Ma et al., 2021). The gaps between the remaining contigs were closed using GapCloser and GapFiller, with purified genomic DNA as the template (Nadalin et al., 2012; Xu et al., 2020). Open reading frames (ORFs) were predicted by GeneMarkS (<http://topaz.gatech.edu/GeneMark/>) and RAST (<http://rast.nmpdr.org/>). The protein function and homology of ORFs were predicted through the BLASTP-algorithm and Hidden-Markov-Module (HMM) based algorithm against NR (2023/02) and Pfam (v.35) database with $1e-5$ as the E-value threshold (Jacob et al., 2008; Katoh and Standley, 2013; Mistry et al., 2013; Mistry et al., 2021). The genomic organization diagram was visualized by the CLC Main Workbench 20. The tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscan-SE/>) was used to detect tRNA sequences in the viral genome (Chan et al., 2021). The complete viral genome sequence of vB_PhoS_XC is now available to access under the accession number MT002874.2 in GenBank. The auxiliary metabolic genes (AMGs) were blast through the Integrated Microbial Genomes/Virus 4 database with the E-value $1e-5$, high search quality genes were chosen for homologous genes, and details were given in [Supplementary Table 1](#).

Metagenome, metatranscriptome sequencing and analysis

Total viral DNA was extracted using QIAamp DNA MiniKit (QIAGEN) according to the manufacturer's instructions. Library construction and high-throughput sequencing were carried out by Novogene (Tianjin, China) using Illumina NovaSeq 6000 (pair-end sequencing, 2×150 bp). Raw reads were quality-controlled by removing the adapters using Cutadapt (Kechin et al., 2017). The high-quality paired-end reads were filtered by Perl scripts following these criteria: 1) without N; 2) no more than 20% bases with a quality score less than 20 (Q20); 3) no more than 30% bases with a quality score less than 30 (Q30) (Yang et al., 2019). To calculate the relative abundances of the vB_PhoS_XC in all meta-omics samples, clean reads were mapped to the vB_PhoS_XC contig using the contig section of CoverM v0.6.1 (<https://github.com/wwood/CoverM>) with default parameter. The relative abundance of the vB_PhoS_XC was quantified as reads per kilobase million mapped reads (RPKM) (Zhang et al., 2023). We also calculated the relative abundance of other representative phages abundant in the ocean including those infecting *Pelagibacter* (Zhao et al., 2013; Chen et al., 2019), *Cyanobacteria* (Labrie et al., 2013), and all *Pseudoalteromonas* which belong to the same subfamily as vB_PhoS_XC (Table 1).

Total RNA extraction, library construction and next-generation sequencing of viral RNA was carried out by Novogene (Tianjin, China) using Illumina NovaSeq 6000 (pair-end sequencing, 2×150 bp). Clean reads were picked from the raw reads by Novogene. Clean reads were then quality trimmed using Trimmomatic v0.39 (Bolger et al., 2014), and then were dropped below SLIDINGWINDOW:4:30, MINLEN:70. The potential rRNA-associated reads were removed using the SortMeRNA v4.3.2 against all databases (Kopylova et al., 2012). Then we got the

TABLE 1 The genome information of *Pseudoalteromonas* phages belonging to the same subfamily as *Pseudoalteromonas* phage vB_PhoS_XC.

Name	Accession number	Length (bp)	G+C content (%)	Number of ORF	Number of tRNA	Proposed subfamily/genus	Viral family
XC	MT002874	46609	40.01	76	1	SG15	Siphoviridae
Station137_DCM_ALL_assembly_NODE_68	Station137_DCM_ALL_assembly_NODE_68	45654	39.53	91	1	SG15	Siphoviridae
DTR_889654	DTR_889654	45599	39.52	91	1	SG15	Siphoviridae
vB_PspS-H40/1	KU747973	45306	40.15	73	0	SG15	Siphoviridae

high-quality reads. The relative abundances of the genes encoded by vB_PhoS_XC were calculated on the basis of all meta-omics samples through CoverM v0.6.1 with default parameter (Salazar et al., 2019). The relative abundances of these genes were then determined as reads per kilobase million mapped reads (RPKM).

Correlation analysis of the phage vB_PhoS_XC with environmental parameters

Temperature, salinity, Dissolved Oxygen (DO) and pH were measured at the sampling site using a YSI proplus multi-parameter water quality analyzer (YSI, Yellow Springs, OH, USA). Seawater filtered through membranes, The concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) were measured with a QuAatro nutrient auto analyzer (Seal Analytical Ltd., King's Lynn, UK). Picoplankton (Pico), *Synechococcus* (Syn), Bacterioplankton (Bac) counting using flow cytometry (Beckman Coulter Inc, USA). All environmental parameters described in Supplementary Table S2. Pearson correlation coefficient (R-value) was used to describe the correlation between vB_PhoS_XC and environmental parameters, including biological parameters (such as Pico, Syn, Bac) and non-biological parameters (such as NH_4 : Ammonium ($\text{NH}_3\text{-N}$), Nitrite ($\text{NO}_2\text{-N}$), Phosphate (PO_4^{3-}), pH value, Nitrate ($\text{NO}_3\text{-N}$), Salinity and Dissolved Oxygen (DO)). The analysis was performed by psych and vegan which are both powered by R (Dixon, 2003).

Result and discussion

Morphological and biological properties of phage vB_PhoS_XC

A novel marine phage, vB_PhoS_XC, was isolated from the coastal waters of Qingdao during an *U. prolifera* bloom (Figure 1A). By the 16srRNA analysis, we determined that the host of the phage vB_PhoS_XC is *Pseudoalteromonas hodoensis* strain H7 (Supplementary Figure S1). Characterization of vB_PhoS_XC revealed an icosahedral head ($\sim 51 \pm 1$ nm in diameter) with a long non-contractile tail ($\sim 86 \pm 1$ nm) (Figures 1B, C), combined with previous research, indicating that it could be classified into the Sipovirus subfamily (Zheng et al., 2023).

The phage vB_PhoS_XC demonstrated a stable titer across a range of pH levels, from 4 to 10. While it could withstand environments of slightly lower (pH3) and higher (pH10-12) acidity, damage did occur in both cases (Figure 2A). Moreover, the phage displayed a more resistance to acidic environments over alkaline. In addition, thermal stability was observed from -20°C to 45°C , although a rapid decrease in phage titer occurred at temperatures of 55°C or higher, with a maximum tolerance of 65°C (Figure 2B). Overall, the phage vB_PhoS_XC displayed a wide range of tolerance to pH and temperature.

The one-step growth curve of phage vB_PhoS_XC demonstrated a latent period of approximately 45 minutes,

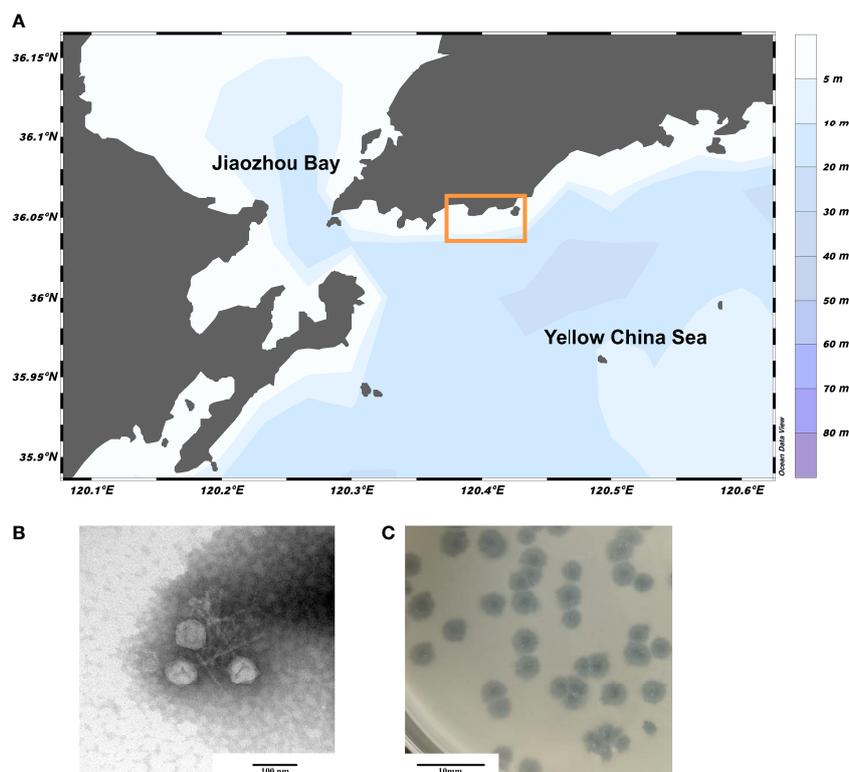


FIGURE 1

Sampling area of Qingdao and Morphology of phage vB_PhoS_XC. (A) Sampling region bounded by the yellow box. (B) Transmission electron micrograph (TEM) of *Pseudoalteromonas* phage vB_PhoS_XC. The scale bar is 100 nm. (C) The phage plaques of *Pseudoalteromonas* phage vB_PhoS_XC. The scale bar is 10 mm.

reaching a plateau stage after 120 minutes (Figure 2C). The final count of released phage particles was 5.0×10^{12} PFU/mL, indicating a burst size of approximately 241 virions per cell. This suggests that VB_PHOS_XC has a lytic activity and a relatively short replication cycle (Yuan et al., 2022).

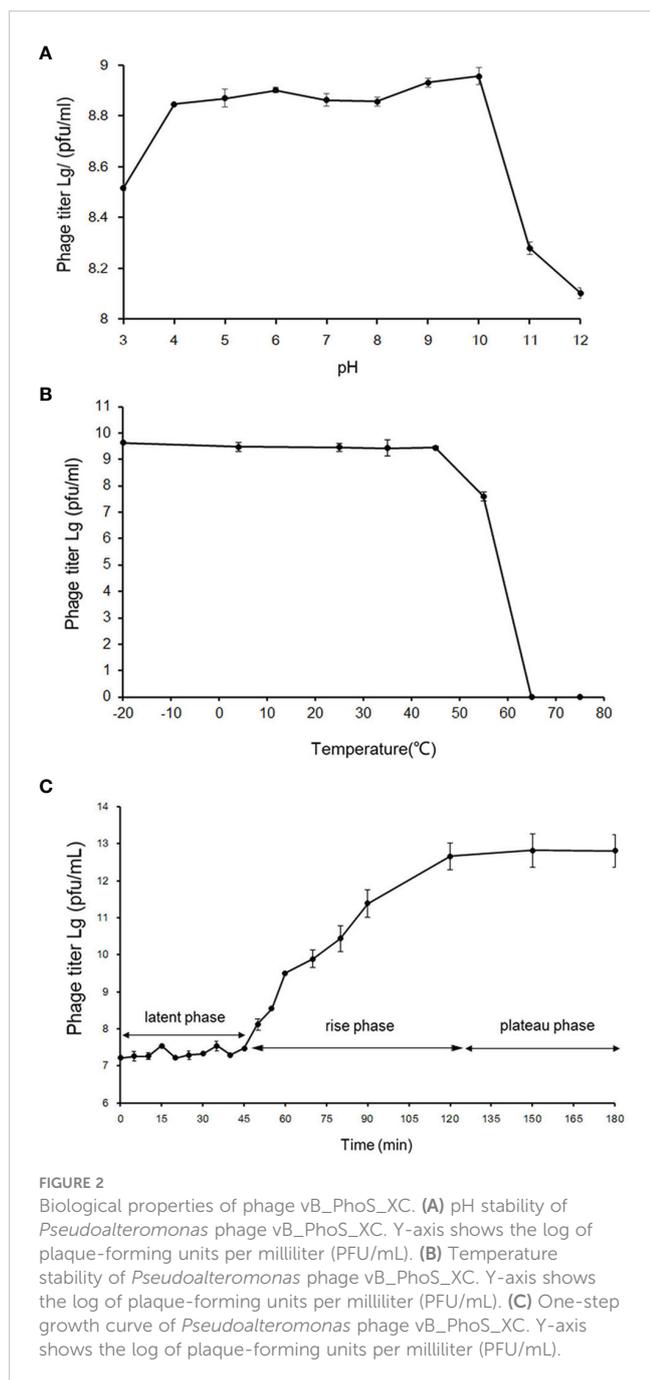
Overall genome features of the phage vB_PhoS_XC

The genome of the phage vB_PhoS_XC consisted a double-stranded DNA (dsDNA) molecule with a GC content of 40.0% and a total length of 46,490 bp (Figure 3). The genome contained 72 open reading frames (ORFs) and one tRNA. Of the ORFs, 37 had no homologous proteins in current public databases, and 35 had known functions, which were grouped into three functional modules: DNA replication and nucleotide metabolism, phage packing and lysis, and phage structure (Table 2). In these 35 known genes, two auxiliary metabolic genes (AMGs) were also predicted to be associated with its host (ORF 11 and ORF 51).

Phage structure and packing genes of the phage vB_PhoS_XC

Most of the ORFs associated with the phage structure in the VB_PHOS_XC genome. In addition to common structure proteins

of *Pseudoalteromonas* phage, the genome contained two unique ORFs associated with Siphovirus structure proteins, namely Tail Tape Measure Proteins (TMPs; ORF 65 and ORF 66). This family of proteins is characterized by short repeats and its function is related to the assembly of the phage, as it participates in determining tail length (Pedersen et al., 2000). TMPs are also known to assist in phage genome delivery into the host cell cytoplasm by interacting with a cognate receptor on the cells surface (Mahony et al., 2016). Studies on the mechanisms of host infection by the Myoviridae phage T4 and Siphoviridae phage T5 have revealed the role of TMP in forming a transmembrane channel that allows the passage of phage DNA into the cell cytoplasm (Boulanger et al., 2008; Hu et al., 2015). Moreover, it has been found that the TMP is a multifunction protein responsible for both tail length determination and viral genome delivery. Consequently, the bacteriophage vB_PhoS_XC containing a TMPs may be used as a model to further investigate the mechanism of *Pseudoalteromonas* infection. Genes related to the packing of the genome in the vB_PhoS_XC were identified. The terminase, composed of two unique subunits (TerS and TerL), was found to be responsible for this process (Esterman et al., 2021). TerS (ORF 35) was found to be responsible for recognizing and binding to the packaging initiation site, as well as regulating the ATPase activity of the TerL (Dixit et al., 2019; Lokareddy et al., 2022b). Additionally, TerL (ORF 36) acted as an ATP-driven molecular motor that transferred viral DNA into the capsid regions, and as an endonuclease that cut the viral genome from the concatemer



(Esterman et al., 2021; Lokareddy et al., 2022a). Finally, these two genes were docked on the portal protein (ORF 8) to facilitate the generation of circular phage heads (Al-Zahrani et al., 2009).

Phage metabolic genes of the phage vB_PhoS_XC

Analysis of the vB_PhoS_XC genome revealed the presence of genes related to some important metabolic processes. ORF 2 was predicted to encode a member of the M15 family metalloproteinase, which is involved in bacterial cell wall biosynthesis and metabolism (Rawlings and Barrett, 2004). Additionally, ORF 15 was observed to

encode a phosphoadenosine phosphosulfate (PAPS) reductase enzyme, a member of sulfonucleotide reductases (SRs) that catalyzes the reduction of adenylated sulfate to sulfite and an essential part of the cysteine biosynthesis pathway (Chartron et al., 2007). PAPS reductase was an important enzyme found in many organisms and played a crucial role in bacteria as it is involved in the metabolism of sulfur-containing compounds such as cysteine and methionine (Kredich and Tomkins, 1966; Chartron et al., 2007). PAPS reductase regulates these compounds by reducing PAPS to 3'-phosphoadenosine-5'-phosphate (PAP). This process involves NADPH (nicotinamide adenine dinucleotide phosphate) as a cofactor and helps maintain proper levels of sulfur-containing compounds within bacterial cells (Murray-Rust et al., 2001).

ORF 17 encodes a DNA-binding transcriptional regulator, which regulates gene transcription by recognizing promoters and enhancers near the transcription initiation site (Wang et al., 2013). ORF 18 is a protein with the catalytic structural domain of GIY-YIG, used to catalyze phosphodiester bond breaking, DNA repair, recombination and genetic element transfer (Truglio et al., 2005; Dunin-Horkawicz et al., 2006). ORF 19 is a DEAD/DEAH box helicase belonging to the family of SvB_ValS_NF2, containing sequence motifs similar to those of DNA helicase proteins; involved in various aspects of transcriptional regulation, recombination and DNA repair (Xu et al., 2021). Additionally, Exodeoxyribonuclease VIII (ORF 25) acts to break dsDNA on both 5' ends, allowing kinked and abnormal portions of the genome to be straightened and repaired through homologous recombination. Interestingly, Exodeoxyribonuclease VIII facilitates genomic repair even in low energy environments and remains stable (Sharma et al., 2019; Kumar et al., 2021).

ORF 28 encodes a bifunctional DNA primase-polymerase, which is widespread in diverse bacteria, archaea, and viruses (Lipps et al., 2003; Geibel et al., 2009; Halgasova et al., 2012). This enzyme has the capacity to act as a primase and a polymerase, and can synthesize long DNA from dNTPs without a preexisting primer bound to the DNA template (Zhu et al., 2017). ORF 57 is predicted to encode a DNA methylase that modifies the DNA of the phage, ultimately avoiding degradation of enzymes from the host cell, as well as protecting phage marked with methylation from its own nucleases which degrade host DNA during the early period of infection (Aravind et al., 2013). Therefore, it may play an important role in the lytic life cycle of the phage (Aravind et al., 2013). The phage vB_PhoS_XC has a relatively well-developed functional composition of the genome. In addition to structural, packing and metabolic genes, some other genes also contribute to its independent survival strategy, such as DNA polymerase (ORF 21), Nucleic acid-binding (ORF22), transcriptional regulator (ORF 27) and ATPase (ORF 40) and most nucleotide metabolism genes.

Two phosphatase genome-biosynthetic-related AMGs

Two host-derived auxiliary metabolic genes (AMGs) were identified in the genome of phage vB_PhoS_XC: a nucleotide

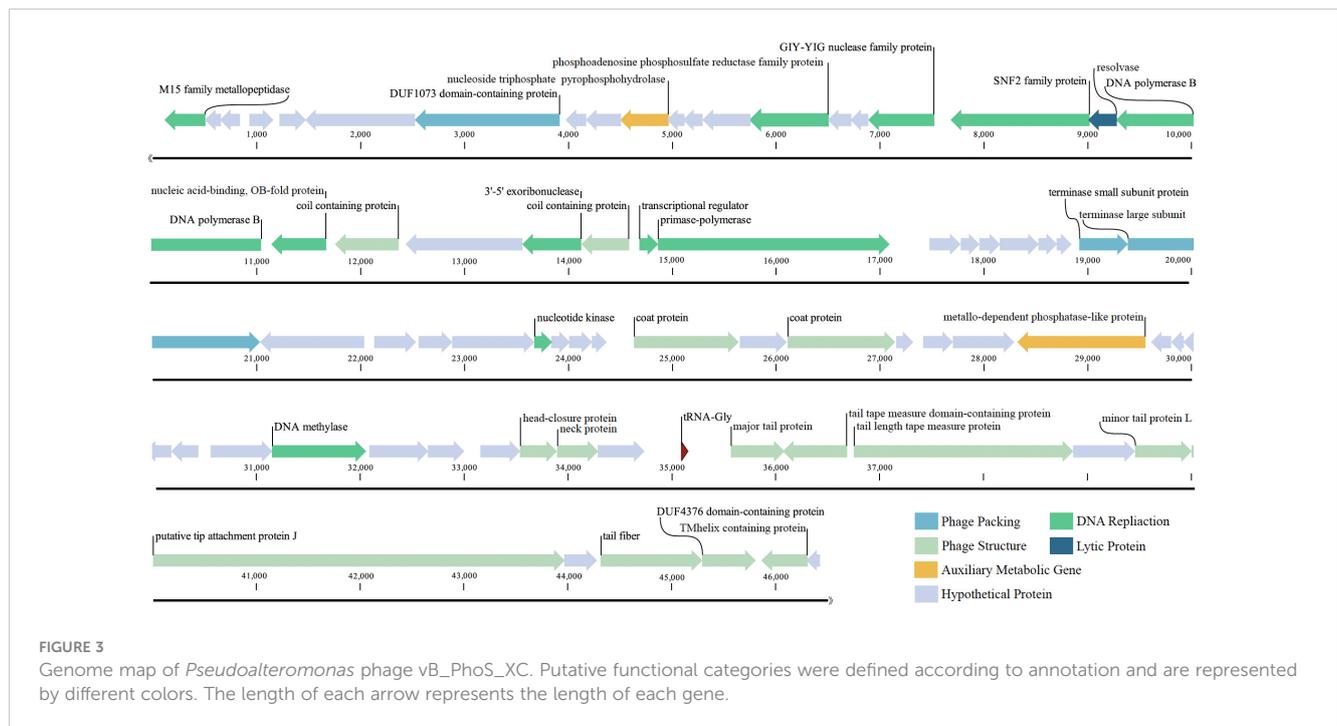


TABLE 2 The conserved domains information of *Pseudoalteromonas* phage vB_PhoS_XC genome.

ORF	Start	Stop	Strand	Function	Match phage	Accession	E value
1	35084	35154	+				
2	504	112	-	M15 family metallopeptidase	<i>Pseudoalteromonas</i> sp.	MCP4060181.1	2e-37
7	2541	1468	-	putative minor head protein	<i>Pseudoalteromonas</i> phage vB_PspS-H40/1	YP_010655240.1	0
8	3916	2522	-	portal protein	<i>Pseudoalteromonas</i> phage vB_PspS-H40/1	YP_010655241.1	0
11	4966	4499	-	nucleotide pyrophosphohydrolase	<i>Pseudoalteromonas</i> phage TW1	YP_010655192.1	1e-36
15	6502	5750	-	phosphoadenosine phosphosulfate reductase family protein	<i>Vibrio</i> phage 1.047.O_10N.286.55.F2	AUR84066.1	2e-138
17	6886	6716	-	DNA-binding transcriptional regulator	<i>Oceanospirillum</i> phage vB_OliS_GJ44	QSM00634.1	6.00e-10
18	7523	6888	-	GIY-YIG nuclease family protein	<i>Pseudomonas</i> paracarnis	WP_220381685.1	1e-32
19	9018	7681	-	DEAD/DEAH box helicase	<i>Pseudoalteromonas</i> phage AL	QIG62466.1	0
20	9280	9005	-	resolvase	<i>Pseudoalteromonas</i> phage PHS21	AQN32316.2	1e-57
21	11048	9273	-	putative DNA polymerase	<i>Pseudoalteromonas</i> phage AL	QIG62464.1	0
22	11667	11140	-	nucleic acid-binding, OB-fold protein	<i>Vibrio</i> phage 1.017.O_10N.286.55.C11	AUR82036.1	2e-68
23	12365	11754	-	coil containing protein	<i>Vibrio</i> phage 1.133.O_10N.222.51.E4	AUR89805.1	3e-31
25	14123	13557	-	exodeoxyribonuclease VIII	<i>Pseudoalteromonas</i> phage PHS21	AQN32313.1	3e-106
26	14584	14126	-	coil containing protein	<i>Vibrio</i> phage LP.1	AZU97906.1	2e-07
27	14683	14862	+	transcriptional regulator	<i>Pseudoalteromonas</i> phage AL	QIG62460.1	9e-22
28	14862	17090	+	bifunctional DNA primase/polymerase	<i>Vibrio</i> phage LP.1	AZU97904.1	0
35	18918	19385	+	terminase small subunit protein	<i>Cupriavidus</i> lacunae	WP_208647650.1	3e-39

(Continued)

TABLE 2 Continued

ORF	Start	Stop	Strand	Function	Match phage	Accession	E value
36	19385	21028	+	phage terminase large subunit	Candidatus Enterovibrio escacola	WP_150137941.1	0
40	22880	23671	+	ATPase	Pseudoalteromonas phage vB_PspS-H40/1	YP_010655271.1	7e-35
45	24631	25638	+	coat protein	Pseudoalteromonas phage H103	YP_009200228.1	1e-55
46	25650	26099	+	head decoration	Pseudoalteromonas phage H103	YP_009200227.1	4.00e-85
47	26110	27144	+	virion structural protein	Pseudoalteromonas phage H103	YP_009200226.1	0
51	29555	28323	-	metallo-dependent phosphatase-like protein	Vibrio phage 1.106.O_10N.286.51.F7	AUR87948.1	4e-141
57	31145	32050	+	DNA methylase	Vibrio phage 1.160.O_10N.261.48.B11	AUR91427.1	6e-173
58	32083	32649	+	minor head protein	Pseudoalteromonas phage vB_PspS-H40/1	YP_010655287.1	1.00e-68
61	33532	33888	+	head-closure protein	Vibrio phage 1.122.A_10N.286.46.F8	AUR89308.1	4e-05
62	33890	34285	+	neck protein	Vibrio phage 1.076.O_10N.286.51.B7	AUR85547.1	3e-16
64	35564	36079	+	major tail protein	Pseudoalteromonas phage vB_PspS-H40/1	YP_010655294.1	9e-110
65	36683	36066	-	tail tape measure domain-containing protein	Pseudoalteromonas phage XCL1123	QGI84348.1	3e-32
66	36747	38858	+	tail length tape measure protein	Pseudoalteromonas phage vB_PspS-H40/1	YP_010655297.1	0
68	39455	40000	+	minor tail protein L	Pelagibacter phage HTVC112P	QZ18222.1	3e-21
69	40000	43962	+	putative tip attachment protein J	Prokaryotic dsDNA virus sp.	QDP59295.1	0
71	44309	45286	+	tail fiber protein	Alteromonas phage vB_AmeM_PT11-V22	YP_009855710.1	3e-08
72	45286	45801	+	tail fiber protein	Pseudoalteromonas phage vB_PspS-H40/1	YP_010655232.1	2e-15
73	46303	45860	-	TMhelix containing protein	Vibrio phage LP.2	AZU97851.1	1e-26

pyrophosphohydrolase gene (ORF 11) and a metallo-dependent phosphatase-like protein gene (ORF 51). These genes were found to contribute to enhanced viral replication through regulation of the host metabolism (Hurwitz and U'Ren, 2016).

ORF 11 encodes nucleotide pyrophosphohydrolase (NTPPHase), a protein found in many marine bacteriophages, suggesting it has an important role in phage proliferation (Bryan et al., 2008; Duhaime et al., 2011). Studies on bacteria have shown NTPPHase is involved in stress responses and removal of noncanonical nucleotides (Lu et al., 2010; Gonçalves et al., 2011), potentially improving the survival of its host in nutrient-depleted environments (Duhaime et al., 2011; Kang et al., 2013; Wang et al., 2021). In addition, ORF 51 encodes a metallo-dependent phosphatase-like protein (PPM). This protein was highly conserved in its sequence and structure with homologous genes identified from prokaryotes, animals, and plants (Kamada et al., 2020). It also regulates the activity of other proteins involved in glycolysis and amino acid biosynthesis (Kamada et al., 2020). Evidence also indicates that PPM has an important role in signaling processes, such as proliferation, apoptosis and

metabolism (Tamura et al., 2006; Lammers and Lavi, 2007, Lu and Wang, 2008). As the viral AMG, it is particularly crucial for bacterial survival and growth under certain conditions (Aravind and Koonin, 1998). Furthermore, its homologs were also found to facilitate viral replication by manipulating host cell metabolism (Wang et al., 2021).

Abundance and ecological function of phage vB_PhoS_XC

The temporal abundance of the phage vB_PhoS_XC was characterized in several viral metagenome data from the coastal seawaters of Qingdao in 2020 year. After analysis of viral community structure in each viral metagenome and together with other common ocean bacteriophages. The results revealed that the relative abundance of Pelagibacter phages was consistently high because it was the most abundance phages in the ocean (Zhao et al., 2019), while the relative abundance of Pseudoalteromonas vB_PhoS_XC phage increased in July (Figure 4). This finding

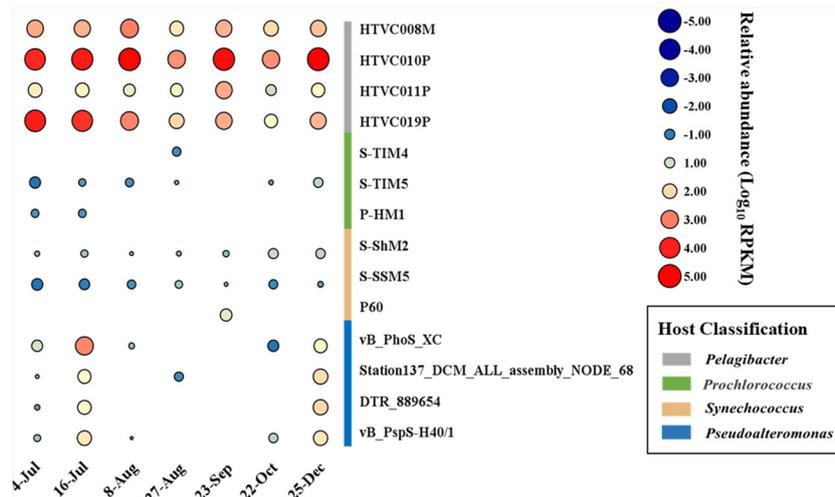


FIGURE 4

Temporal changes of *Pseudoalteromonas* phage vB_PhoS_XC. Bubble size shows relative abundance on *Pseudoalteromonas* phage vB_PhoS_XC of different time series, expressed by RPKM (reads per kilobase per million mapped reads) values.

correlated with an observed outbreak and extinction process of *U. prolifera* green tide in the seawater region of Qingdao in the same time period (Figure 5A). This suggests that the increase in availability of *Pseudoalteromonas* species likely resulted in the corresponding increase in abundance of its specific phage vB_PhoS_XC. These results demonstrate the dynamic relationship between prokaryotic and eukaryotic components of the coastal ecological system. The abundance of this phage significantly increased might be with the accumulation of high amounts of algal-derived DOM after the bloom arrival (Han et al., 2023). By the end of the bloom termination stage, most of the labile DOM had been utilized by bacteria, it might result the decrease of viral abundance like phage vB_PhoS_XC. Although there was no obvious correlation between the phage vB_PhoS_XC and environment factors (Supplementary Figure S2), further implied that there might be a complex interaction between the phage vB_PhoS_XC and its host.

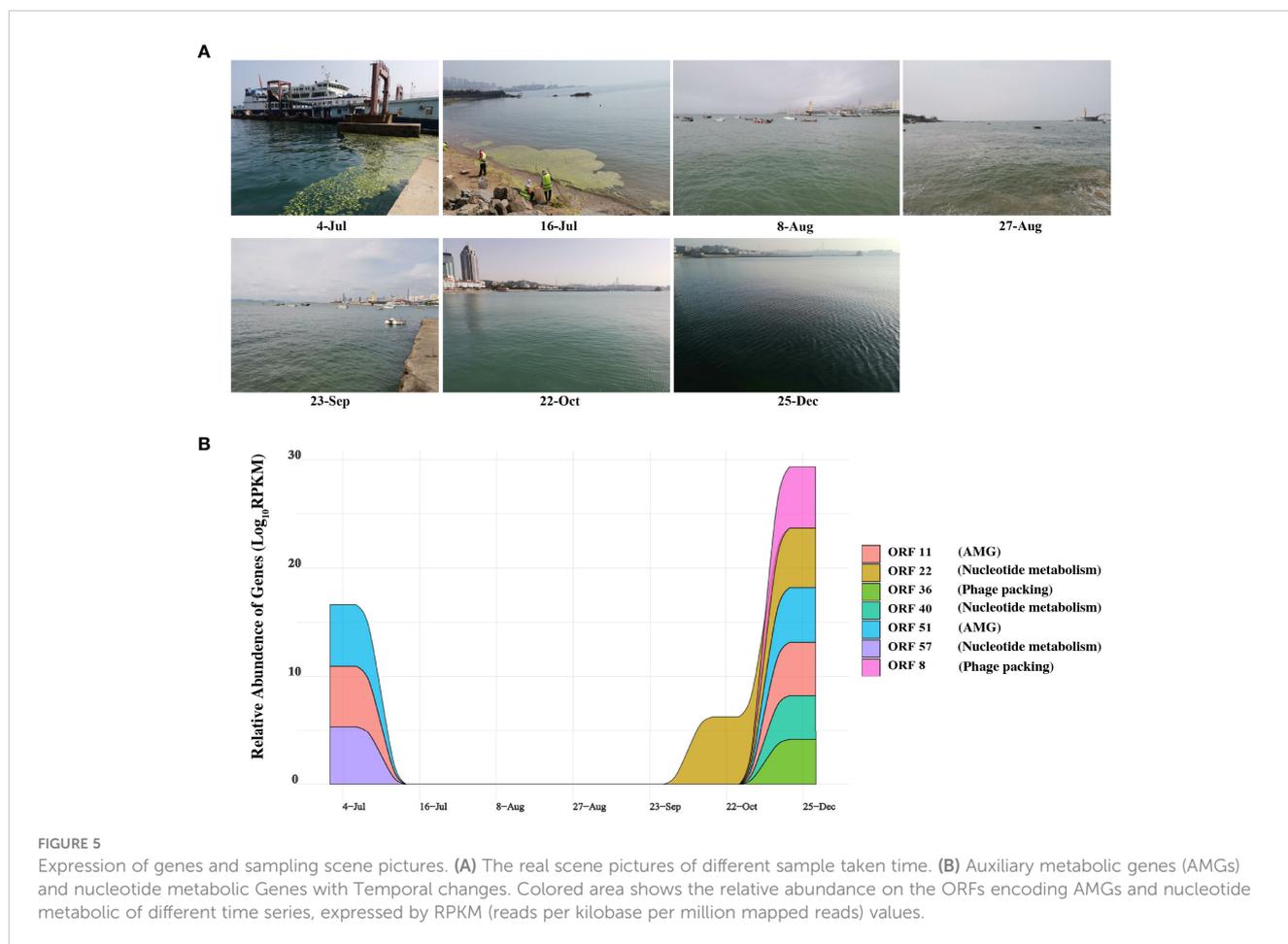
Thus, we also analyzed the expression of AMGs and other functional genes in the corresponding metatranscriptome data to explore the correlation between the phage vB_PhoS_XC and its host. Figure 5B demonstrates that ORF 11 and ORF 51 and ORF 57 (AMGs or accessory metabolic genes) had a high relative abundance in the phage on July 4th and December 25th (Figure 5B). ORF 11 is largely involved in proliferation, stress response, and increasing the survival rate of the host. This may assist the host cells in preventing programmed cell death resulting from rapid changes in abiotic factors caused by *U. prolifera* blooms. ORF 51 regulates the activity of proteins associated with a variety of metabolic pathways. Every year from June to July, a large-scale *U. prolifera* bloom typically occurs in Qingdao. This bloom rapidly causes a decline in inorganic carbon, increasing the pH of the seawater (Han et al., 2023). When

the bloom terminates, pH, DOM, DOC, and nutrients are significantly altered to an extent that disrupts the dynamic balance of bacterial community structure and function (Li H et al., 2016; Zhang and Wang, 2017; Chen et al., 2020; Liang et al., 2021). Environmental changes result in the increased expression of these AMGs, which helps the host to survive and maximizes their own replication.

Interestingly, the relative abundance of some genes had a higher expression in December (ORF 22, ORF 36, ORF 40, ORF57 and ORF 8) (Figure 5B). At this time, concentration of nutrients is poor, the water temperature is low and the abundance of bacterioplankton are significantly reduced (Uyà et al., 2017; Guo et al., 2020; Zhao et al., 2022). We speculated that during this period the phage vB_PhoS_XC is manipulating the host through AMGs to improve metabolic efficiency and survival. It may also improve its replication efficiency by repacking genes and increasing nucleotide metabolism, helping create a “virus factory” in the host cells to maximize the output when favorable conditions return.

Conclusion

Isolating and culturing viruses that infect bacteria provides a new and valuable insight into novel viral sequences from metagenomic and metatranscriptomic data. Few *Pseudoalteromonas* phages have been isolated from the bacterial hosts and research on them is limited. This study aimed to isolate the phage vB_PhoS_XC from coastal seawaters of Qingdao during a *U. prolifera* bloom. Results indicate that the phage has a short latent period and a large burst size, as well as being active in a wide range of environmental conditions. Additionally, abundance of the phage was observed to be higher



during *U. prolifera* blooms and winter, two AMGs and nucleotide metabolism system were also expressed a higher abundance in these periods. This suggests that the phage may be able to improve the host's survival rate and enhance its metabolic efficiency to improve its own replication. Consequently, further research will enable a better understanding of the role of phages in marine ecosystems, as well as their interactions with their hosts.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.cncb.ac.cn>, GSA: CRA010505.

Author contributions

YTL, AM and MW: conceptualization, revision, project administration, supervision and funding acquisition. JS, XZ, KZ, MH, GL, YDL and FK: methodology, formal analysis, writing, and original draft preparation. JS, XZ and GL: sample collection and expedition organization. YS, WM, LW and FK: review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1201434/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Phylogenetic tree of the *Pseudoalteromonas* genus.

SUPPLEMENTARY FIGURE 2

Pearson correlation coefficients among the phage vB_PhoS_XC, Picoplankton (Pico), Synechococcus (Syn), Bacterioplankton (Bac), NH₄: Ammonium (NH₃-N), Nitrite (NO₂-N), Phosphate (PO₄³⁻), pH value, Nitrate (NO₃-N), Salinity and Dissolved Oxygen (DO). *Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, *** Correlation is significant at the 0.001 level.

SUPPLEMENTARY TABLE 1

Homologous genes of auxiliary metabolic genes (AMGs).

SUPPLEMENTARY TABLE 2

The sampling environmental parameters.

FILTER_NO_SINGLE.PL.ZIP

The perl script can be used for removing N from the data.

FILTER_NO_SINGLE2030.PL.ZIP

The perl script can be used for removing less than 20% and 30% bases with a quality score.

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