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Genomic and biological characterization of the *Vibrio alginolyticus*-infecting "*Podoviridae*" bacteriophage, vB_ValP_IME271

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Abstract

As an opportunist pathogen, *Vibrio alginolyticus* (*V. alginolyticus*), causes disease in marine animals. Bacterial contamination of seafood is not uncommon, and phage therapy is considered a safe way to decontaminate such foods to control the emergence of vibriosis. Here, we report on the isolation of a new, virulent phage called vB_ValP_IME271 (designated phage IME271), which infects *V. alginolyticus* and was isolated from seawater. Phage IME271 displayed good pH (7–9) and temperature tolerance (< 40 °C) and had a broad host range against Vibrio isolates, including 7 strains of *V. alginolyticus* and11 strains of *V. parahaemolyticus*. The IME271 genome was sequenced and annotated, the results of which showed that this phage is a *Podoviridae* family member with a genome length of 50,345 base pairs. The complete genome is double-stranded DNA with a G+C content of 41.4%. Encoded within the genome are 67 putative proteins, of which only 22 coding sequences have known functions, and no tRNAs are present. The BLASTn results for IME271 showed that it only shares similarity with the Vibrio phage VPp1 (sequence identity score of 96% over 87% of the genome) whose host is *V. parahaemolyticus*. Comparative analysis showed that IME271 and VPp1 share a similar genomic structure, and the structural proteins are highly similar (> 95% similarity score). In summary, our work identified a new lytic *Podoviridae* bacteriophage, which is infective to *V. alginolytic*us and *V. parahaemolyticus*. This bacteriophage could potentially be used to control *V. alginolyticus* and *V. parahaemolyticus* infections in marine animals.

Keywords *Vibrio alginolyticus* · Bacteriophage · Biological characteristics · Genome

Introduction

In line with the development of the maricultural industry, there has been an increase in the frequency of diseases found in fish, notably, *Vibrio* infections. *V. alginolytic* can cause *vibriosis*—a term for a group of well-known fish diseases reported from a large number of marine fish species [1]. Each year, the diseases associated with *Vibrio* cause significant economic losses in the fishing industry, and there have been world-wide reports of vibrio pathogens such as *Vibrio alginolyticus* [2], *V. parahaemolyticus* [3], *V. harveyi* [4], *V. vulnificus* [5], and *V. fluvialis* [6]. *V. alginolyticus* exists in many marine animals within the normal marine microbiota but is a conditional pathogen of several animals including fish, shrimps, and other shellfish. As a mesophilic bacterium, *V. alginolyticus* is most pathogenic to breeding animals increases during the summer. Infections often occur when the immune function of the target animal is compromised or the environment becomes polluted [7]. Additionally, it has

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been reported that *V. alginolyticus* can cause food poisoning [8] in human.

The indiscriminate use of antibiotics has led to the development of resistant strains of *Vibrio*, which have contributed toward the inefficacy of these agents. Phage therapy is a promising alternative to antibiotics for hindering the growth of pathogenetic bacteria, and bacteriophages, a genetically diverse group of viruses, are abundant in all environments. Lytic phages exhibit a highly potent, species-specific bacteriolytically activities and are potentially useful for the treatment and prophylaxis of bacterial infections [9].

In the present study, we have report on the detailed biological characterization and genomic information relating to vB_ValP_IME271, infect *V. alginolyticus* and *V. parahaemolyticus*. In order to describe the characteristics of phage vB_ValP_IME271, we selected the host when the phage was first discovered to conduct experiments and report the results. The results of this study will provide opportunities for further investigations on this phage in terms of how it could be used in the future to treat *V. alginolyticus* infections in marine animals.

Materials and methods

Bacterial strains and culture conditions

V. alginolyticus 1651, an indicator strain, was isolated from a diseased fish from Huanghai, China, in 2016. *V. alginolyticus* 1651 was stored at − 70 °C in 50% (v/v) glycerol and cultivated at 37 °C, with shaking at 220 rpm, in liquid brain–heart infusion (BHI) broth (Becton Dickinson, Franklin Lakes, NJ, USA). The other strains used in this study were 29 strains of *V. alginolyticus*, 66 strains of *V. parahaemolyticus*, 3 strains of *V. vulnificus*, and 1 strain of *V. fluvialis*, all of which came from the bacteria library at the Beijing Institute of Microbiology and Epidemiology, China. All of the bacterial strains were identified by 16S rRNA sequencing with the following universal primers: 27F: 5′ AGAGTTTGATCMTGGCTAG 3′ and 1492R: 5′ TACGGY TACCTTGTTACGACTT 3′.

Bacteriophage isolation and propagation

Phage vB_ValP_IME271 was isolated from the seawater collected from Huanghai (Qingdao, China), from the host *V. alginolyticus* 1651 strain. Briefly, at least 2 liters of seawater were centrifuged at 10,000 × *g* for 8 min, and the supernatant obtained was filtered through 0.22-μm membranes. The filtered supernatant was placed in a dialysis bag (MD34), which was covered with PEG 8000 powder for 16 h, and the concentrated supernatant in the bag should be retained. The supernatant containing the crude bacteriophage pool is hereafter referred to as 'the sample'. The double-layer agar technique was used to incubate phage. Different phages will show different transparent rings on solid media when cultured with bacteria. The harvested phages were selected according to their individual shapes. Phages displaying large, clear and non-turbid plaques without a halo were classified as virulent [10]. A single isolated plaque was picked from the double-layered plate to start the second round of amplification to ensure that only a single phage was isolated. The purified phage preparation was then stored at 4 °C.

Transmission electron microscopy

The purified phage (10 μL at 10^8 pfu/mL) was directly stained with 2% (w/v) phosphotungstic acid on a carbon-coated grid. Images were examined with a transmission electron microscope (JEM-1200EX, Japan Electron Optics Laboratory Co., Tokyo, Japan) at an acceleration voltage of 120 kV.

Optimal multiplicity of infection (MOI) determination and one-step growth assays

To determine the optimal MOI, serial dilutions of *V. alginolyticus* strain 1651 grown to its exponential phase were added to aliquots of a stock solution of vB_ValP_IME271. According to the proportion of infection, the ratio of 0.01, 0.1, 1, 10, 100. 100 μL of phage and 100 μL of *V. alginolyticus* strain 1651 (1×10^8 CFU/mL) were added to 5 mL of liquid medium, shaken at 37 °C for 10 h, centrifuged at 4000 r/min for 15 min to remove the cells, and the precipitate was removed. The supernatant was filtered through a 0.22-μm membrane, and the titer of the phage was determined by a two-layer plate method. The highest multiplicity of infection was the optimal multiplicity of infection [11, 12].

One-step growth curve experiments were undertaken as described previously [10]. Briefly, a mid-exponential growth-phase culture of *V. alginolyticus* strain 1651at an optical density (OD) 600 of 0.6 was harvested and infected with vB_ValP_IME271 at a MOI of 0.01. Three 150 μL aliquots of cells in culture medium were then collected at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, and 160 min post-infection. The samples were centrifuged at 10,000×*g* for 2 min, the supernatants removed, and the phage titers were determined using the double-layer agar method [13]. Samples were diluted and plated immediately to determine the bacteriophage titers.

Determining the host range of bacteriophage vB_ValP_IME271

The test strains (32 different strains of *V. alginolyticus*, 66 different strains of *V. parahaemolyticus*, 3 different strains

of *V. vulnificus*, and 1 strain of *V. fluvialis*) were individually cultured to an OD_{600} of about 0.6. Aliquots (400 µL) from each culture were individually added to 5 ml of pre-warmed semi-solid liquid medium, mixed, and then poured onto individual plates containing solid medium to form a double-layered plate, and then cultivated at room temperature for 5 min. Next, 2.5 µL of the purified phage solution (10^8 pfu/mL) was dropped onto each double-layered plate and the plates were cultivated at room temperature for 20 min until the phage solution was absorbed. The plates were incubated at 37 °C and then were inspected for the presence of plaques at 9 h post-cultivation.

Sensitivity of bacteriophage vB_ValP_IME271 to ultraviolet (UV) light

The phage solution (20 ml, 10^8 pfu/mL) was poured into individual 8-cm sterile culture dishes and placed under UV irradiation (20 W, 30 cm). Three 100 µL samples were removed at 10, 20, 30, 40, 50, and 60 min, and the double-layer agar method was used to calculate the titer of each sample. A phage not subjected to UV irradiation was used as a control.

Sensitivity of bacteriophage vB_ValP_IME271 to pH

The pH value of the culture medium was set at 2 to 14 using 1 mol/L HCl or 1 mol/L NaOH. At each pH value, 900 µL of the BHI liquid medium was collected for three sets of parallel experiments. To each of these samples, 100 µL of phage solution (10^8 pfu/mL) was added and the samples were cultivated for 60 min at 37 °C, after which the double-layer agar method was used to calculate each sample's titer.

Sensitivity of bacteriophage vB_ValP_IME271 to chloroform

A solution of phage B_ValP_IME271 (10^8 pfu/mL) was mixed with chloroform at 0, 1%, 2%, and 5%, and then incubated for 30 min at 37 °C with shaking. Three 100 µL samples were removed from each chloroform-containing sample, and the double-layer agar method was then used to calculate each sample's titer.

Thermal stability of bacteriophage vB_ValP_IME271

BHI liquid medium (900 µL) was warmed to 40, 50, 60, 70, and 80 °C for 60 min. After the temperature stabilization, 100 µL of each phage solution (10^8 pfu/mL) was added to the samples followed by sample cultivation for 30 or 60 min. After the samples cooled to room temperature, the double-layer agar method was used to calculate each sample's titer.

Genomic DNA extraction

The bacteriophage stock solution (600 µL, 10^8 pfu/mL) was treated with 1 µg/mL of DNase I and 1 µg/mL of RNase A (Thermo Scientific, Waltham, MA, USA) before being incubated overnight at 37 °C to remove host bacterial DNA and RNA. Genomic DNA from vB_ValP_IME271 was extracted using protease K and sodium dodecyl sulfate, as described previously [14–16].

Library preparation and genome sequencing

The extracted vB_ValP_IME271 genomic DNA was sequenced using the semiconductor sequencer in an Ion Torrent™ Personal Genome Machine (Life Technologies, Carlsbad, CA, USA) [17]. This technology uses emulsion PCR and a sequencing-by-synthesis approach [18]. Library fragment preparation, fragment immobilization, clonal amplification, and sequencing reactions were performed according to the Ion Torrent™ sequencing protocols. The H^+ Ion Torrent™ signal was detected during the sequencing-by-synthesis process. The ~300-bp reads were assembled using the Newbler version 3.1 *de novo* assembly program (Roche, Basel, Switzerland) with default parameters.

Whole genome sequence analysis of phage vB_ValP_IME271

The initial vB_ValP_IME271 gene annotation was performed using the Rapid Annotation Subsystem Technology (RAST) annotation server [19]. The phage classifications were found in the ICTV (The International Committee on Taxonomy of Viruses; https://talk.ictvonline.org/). The evolutionary phylogenetic analysis of the major capsid protein (MCP) was conducted in MEGA6 via the maximum-likelihood ratio UPGMA method with 1000 bootstraps (repeated 1000 times) [20]. All the major capsid protein amino acid sequences from the phages were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Easyfig was used for the comparative genomic analysis of the VPp1 and vB_ValP_IME271 Vibro phages [21]. The genome sequences for VPp1 were downloaded from GenBank. Different similarity identity (95% and 90%) is used to compare similarities and differences between genomes of VPp1 and vB_ValP_IME271, separately.

Results

Morphology of phage vB_ValP_IME271

The vB_ValP_IME271 bacteriophage was isolated from seawater from Huanghai (Qingdao, China) using the indicator

bacteria *V. alginolyticus* strain 1651. The vB_ValP_IME271 bacteriophages formed clear, transparent plaques (Fig. 1a). Following complete lysis of the host cells, the bacteriophage titer in the supernatant was 1.18×10^8 pfu/mL. The results of the transmission electron microscopy revealed that vB_ValP_IME271 had an isometric, pentagonal head of 55 nm in diameter and a short, non-contractile tail, which indicating that it belongs to the *Podoviridae* family (Fig. 1b).

Optimal MOI and one-step growth curve

V. alginolyticus strain 1651, when infected at a MOI of 0.01, generated the maximum bacteriophage titer (Table 1), indicating that 0.01 was the optimal MOI for the vB_ValP_IME271 titer.

The one-step growth curve showed that vB_ValP_IME271 had a 90-min latent period and a 60-min rise phase (Fig. 2a), followed by the plateau phase. The burst size (i.e., the maximum number of progeny released from one host bacterium [22]) was calculated by dividing the phage titer for the rise phase by the original titer from the host $((4.0\times10^9)/(1\times10^8)=40$ pfu/cell).

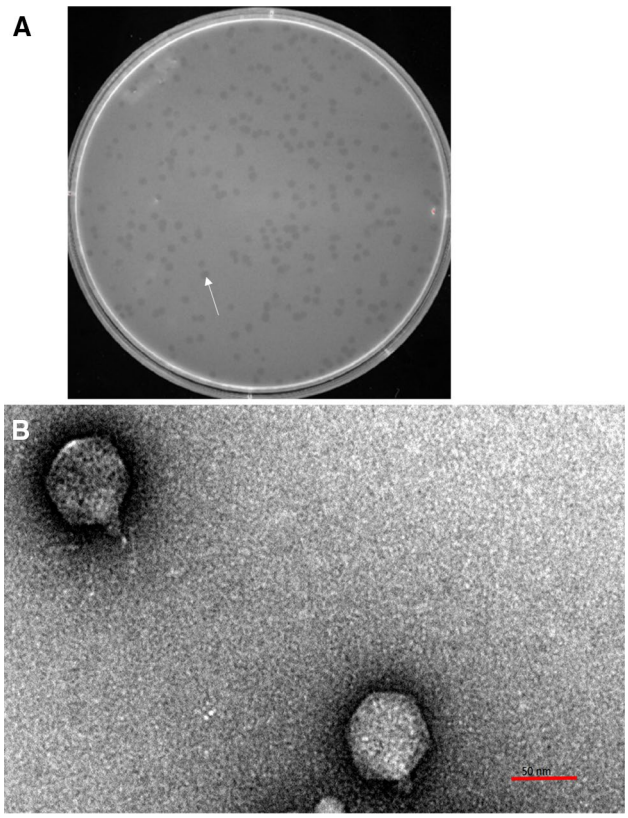

Fig. 1 Morphology of phage vB_ValP_IME271.**a** Phage vB_ValP_IME271plaques. **b** Morphology of bacteriophage vB_ValP_IME271 as revealed by transmission electron microscopy

Table 1 Optimal multiplicity of infection determination

Tube no.	Number of bacteria (cfu)	Number of phage (pfu)	MOI	Titer at 6 h/(pfu/mL)
1	10^8	10^6	0.01	8.7×10^9
2	10^8	10^7	0.1	5.2×10^9
3	10^8	10^8	1	9×10^8
4	10^8	10^9	10	7×10^8
5	10^8	10^{10}	100	6×10^8

Pfu plaque forming units, *MOI* multiplicity of infection

Phage host range analysis

The host range for vB_ValP_IME271 (based on spot testing) was tested for 32 different strains of *V. alginolyticus*, 66 different strains of *V. parahaemolyticus*, 3 different strains of *V. vulnificus*, and 1 strain of *V. fluvialis* (Table S1). The result showed that 12 strains of *V. alginolyticus* and 7 strains of *V. parahaemolyticus* were sensitive to lysis by phage vB_ValP_IME271 (Table S3). No sensitivity was observed in the other test strains (15). The lysis effect (19/102) shows that phage vB_ValP_IME271 has the potential to be a candidate for phage therapy.

Determination of phage survival

After UV irradiation, the survival rate for phage vB_ValP_IME271 decreased sharply over time (Fig. 2b), and following 40 min of UV irradiation, its survival rate was only 6%. After UV irradiation for 50 min, the phage was essentially inactivated, indicating that it is sensitive to this type of radiation.

Every phage has an optimal pH for survival. Phage vB_ValP_IME271 showed an optimal lysis activity at pH 8.0 (Fig. 2c). When the pH level was increased, the titer of phage vB_ValP_IME271 decreased sharply, and at pH 12.0, the phage was essentially inactivated. As the pH decreased from 8, the titer of vB_ValP_IME271 decreased sharply to essentially become inactive at pH 3.0.

As shown in Fig. 2d, with increasing temperature, the titer of phage vB_ValP_IME271 decreased sharply. After 30- and 60-min incubation within a 40–80 °C temperature range, the phage became inactivated at 60 °C. This result indicates that vB_ValP_IME271 is temperature sensitive.

The phage suspension was mixed with 0%, 0.1%, 2%, and 5% chloroform, which corresponding to a final titer of 1.73×10^8, 1.10×10^8, 0.97×10^8, and 1.0×10^8 pfu/mL, respectively. There was no significant difference in the titer between the untreated phages and the chloroform-treated phages, which indicates that phage vB_ValP_IME271 was not affected by chloroform treatment. As chloroform is fat

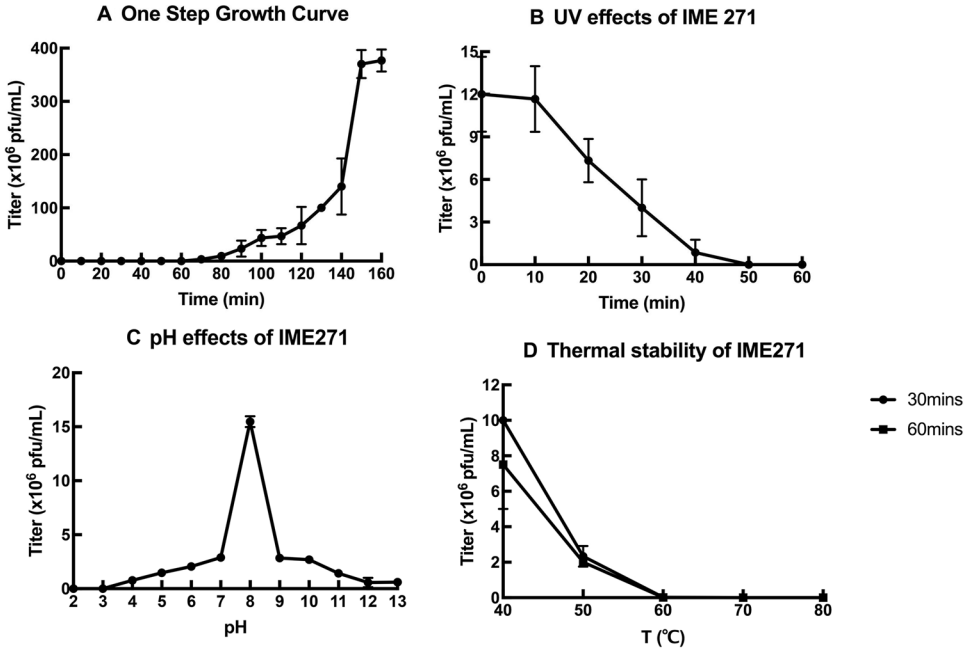

Fig. 2 Phage survival determination. **a** One-step growth curve for phage vB_ValP_IME271. **b** Sensitivity of phage vB_ValP_IME271 to ultraviolet (UV) irradiation. **c** Sensitivity of phage vB_ValP_IME271 to pH. **d** Thermal stability of phage vB_ValP_IME271

soluble, this result suggests that phage vB_ValP_IME271 does not contain lipids [14].

Overview of the phage vB_ValP_IME271 genome

After sequencing the genome of vB_ValP_IME271, the 8, 436, 589 filtered reads (~200 bp/read) we obtained were assembled into one contig, which could be circularized. The complete genome of phage vB_ValP_IME271 is 50,345 bp in length, with an average GC content of 41.4%. The genome sequence comparison using the BLASTn method revealed that the complete genome of this phage had a nucleotide similarity score of 96% over 87% of the genome coverage with the known bacteriophage *Vibrio* phage VpP1 (GenBank accession number KJ936628). The RAST annotation results showed that the phage vB_ValP_IME271 genome contains 67 coding sequences (CDSs) and no tRNAs (Table 2). Among the putative coding sequences, the classifications of the 22 coding sequences with functional annotations are being listed (Table 2), and these CDSs can be divided into three groups that encode structural proteins, metabolic proteins, and replication/packaging-associated proteins. According to the annotation results, there are only seven known structurally related proteins including three phage tail fiber proteins, a phage capsid, and two constituent protein. Only eight proteins are predicted to be replication/packaging-associated: DNA primase/helicase (CDS30), DNA polymerase (CDS32), exonuclease (CDS35), ribonucleotide reductase of class Ia (aerobic), alpha subunit and beta subunit (CDS36 and CDS 37), terminase large subunit (CDS60), and terminase small subunit (CDS65). The portal protein serves at least two critical functions during the phage lifecycle. During infection, the phage genome enters the host cell through the portal protein. During phage assembly, the terminase complex binds to the portal protein to actively package DNA into the capsid [23]. The large and small subunits of terminase are involved in the DNA packaging machinery. The large subunit is responsible for DNA translocation powered by ATP [24].

Phylogenetic tree analysis for phage vB_ValP_IME271

We analyzed the evolutionary relationships for phage vB_ValP_IME271 and other known phages within the *Podoviridae* family, which includes *Autographivirinae, Picovirinae* and unassigned subfamilies. The amino acid sequence of the capsid protein, a conserved sequence among these viruses [25, 26], was used to construct a phylogenetic tree (Fig. 3). We consistently found that phage vB_ValP_IME271 clustered closely with the LUZ24 *Pseudomonas* phage on the phylogenetic tree, the latter of which is now classified as belonging to the *Podoviridae* family.

Comparative genomic analysis of phage vB_ValP_IME271

Because of the high similarity between the phage and vpp1, we performed a detailed comparison of the genomes of phage vB_ValP_IME271 and VPp1 [17]. Our comparative genomic analysis showed that half of vB_ValP_IME271 and VPp1 proteins are hypothetical protein. The genome region containing the structural proteins showed > 95% sequence similarity, suggesting that there may be the similar structural

Table 2 Functional classification of the 22 coding sequences in the vB_ValP_IME271 genome

Category	CDSs	Putative functions
Structural proteins	CDS48	Phage tail fiber protein
	CDS49	Constituent protein
	CDS50	Constituent protein
	CDS51	Phage tail fiber protein
	CDS53	Tail tubular proteins
	CDS56	Phage capsid and scaffold
	CDS59	Phage portal protein
Metabolic proteins	CDS14	Glutamine aminotransferase
	CDS17	Putative amidoligase
	CDS18	ATP-grasp enzyme
	CDS22	Phosphoribosyl-ATP pyrophosphohydrolase-like
	CDS25	Thymidylate synthase thyX
	CDS27	Thymidylate synthase thyX
	CDS64	Endolysin
Replication associated proteins	CDS30	DNA primase/helicase
	CDS32	T7-like phage DNA Polymerase
	CDS35	Phage exonuclease
	CDS36	Ribonucleotide reductase of class Ia (aerobic), alpha subunit
	CDS37	Ribonucleotide reductase of class Ia (aerobic), beta subunit
	CDS40	Endodeoxynuclease
	CDS60	Terminase, large subunit
	CDS65	Terminase, small subunit

CDS coding sequence

proteins making up each phage (Fig. 4a). This result is also according to the phylogenetic tree analysis for phage vB_ValP_IME271, with the phages sharing a high similarity score (> 90% identity) in other areas (Fig. 4b).

Discussion

Many different types of phages have been discovered that target *Vibrio alginolyticus*, and Table S3 shows the general information for 38 *Vibrio* phages. Thus far, each of these *Vibrio* phages has been found to target a different host, and even the same bacterial genus may be targeted by different phage types, such as *Podoviridae, Myoviridae*, and *Siphoviridae*, but most of them are *Podoviridae* members. Phages have diverse host ranges and are also distributed widely across the world (China, USA, Germany, India, Portugal, and Greece, among other countries). However, not all known phages have been characterized and complete information is still lacking for the following strains: *Vibrio* phage VP01 has no genomic information, and *Vibrio* VP-1, *Vibrio* VP-2, and *Vibrio* VP-3 phages have no genomic or morphological information.

Our comparative analysis of Vibrio phage VPp1 [27] and vB_ValP_IME271 has revealed that they have different genomic lengths, G+C contents and numbers of encoded open reading frames (ORFs) (Fig. 4). The amino acid sequence comparison using the BLASTp method revealed that ORF64 from vB_ValP_IME271 had a similarity score of 95% of the amino acid coverage with ORF33 from Vibro phage VPp1(Table S2). According to previous report, ORF33 from Vibro phage VPp1 which encoded endolysin (named LysVPp1) has a broader host range (9/12 *V. parahaemolyticus*) than phage VPp1 (3/12 *V. parahaemolyticus*) [28]. Phage vB_ValP_IME271 both can infect some strains of *Vibrio alginolyticus* and *V. parahaemolyticus*. We also intend to express lysin protein (ORF64) from vB_ValP_IME271 to determine whether the protein it encodes in this phage also has a broader host range.

Compared with any single phage, phage cocktails are highly effective at arresting the entire infection process of their cognate viruses. Hence, deployment of this approach could serve as an effective strategy for arresting the entire infection process [28–31] and could be implemented under the framework of developing personalized phage therapies. A phage selected as a potential biocontrol agent should preferably be a virulent phage with no side effects on human health or that of marine animal. Among the 67 ORFs with a predicted function in vB_ValP_IME271, none are associated with toxicity, allergens or antibiotic resistance. However, as more than half of the predicted ORFs in vB_ValP_IME271 are hypothetical proteins, it

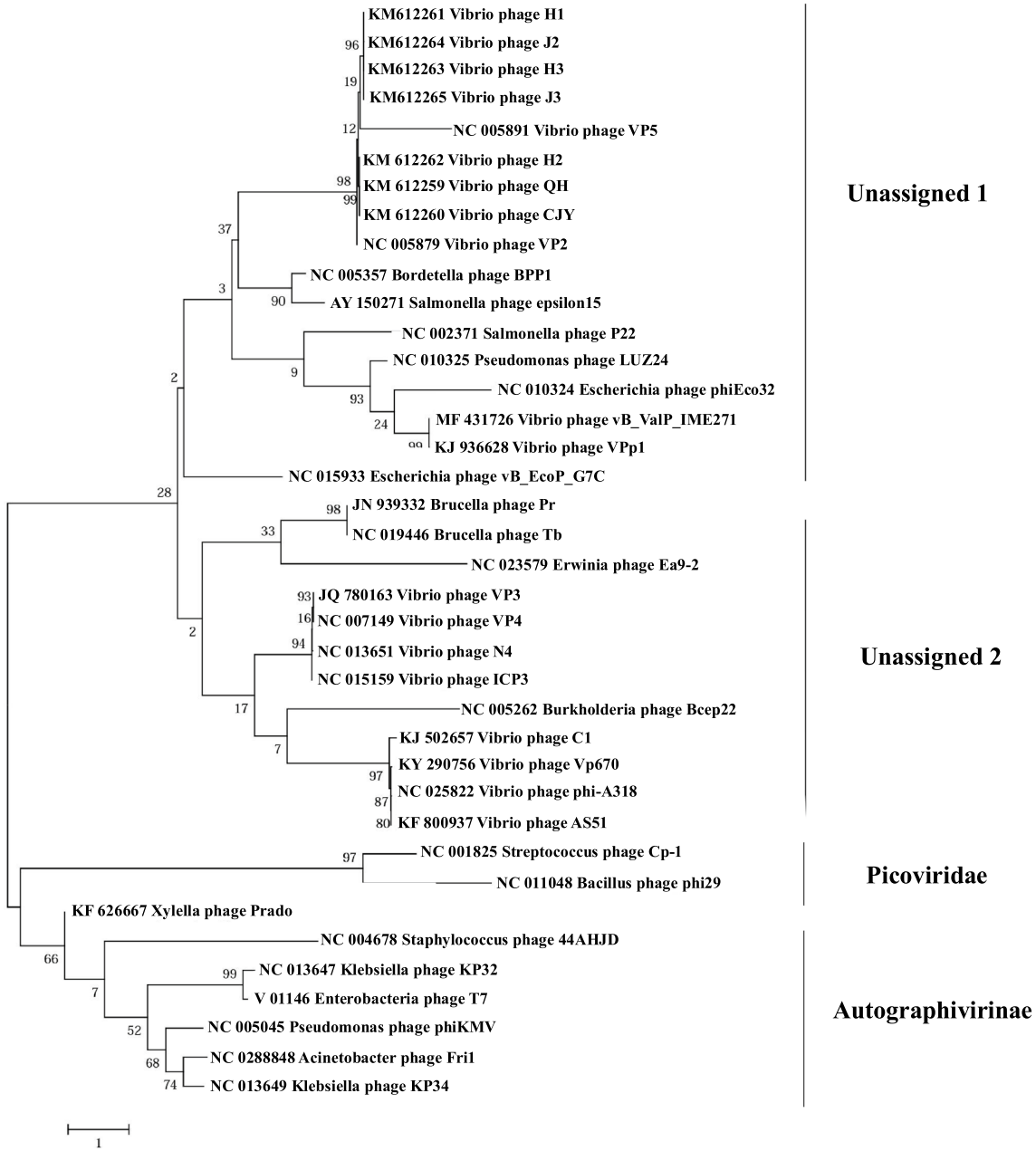

Fig. 3 Phylogenetic analysis of vB_ValP_IME271. A phylogenetic tree was constructed in UPGMA with 1,000 bootstrap replicates. Major capsid amino acid sequences of the investigated phage and other phages that are classified within the *Picovirinae* subfamily were aligned using MEGA6 software and utilizing MUSCLE. Bootstrap support values (numbers on the lines) are indicated for selected internal branches. vB_ValP_IME271 is highlighted by a box. (The GenBank accession numbers of the major capsid proteins are as follows: vB_ValP_IME271: ASR73870.1; Vibrio phage H1:AIZ01468.1; Vibrio phage J2: YP_009152772.1; Vibrio phage H3: AIZ01564.1; Vibrio phage J3: AIZ01660.1; Vibrio phage VP5: YP_053021.1; Vibrio phage H2: AIZ01516.1; Vibrio phage QH: YP_009153047.1; Vibrio phage CJY: KM612260.1; Vibrio phage VP2: YP_024425.1; Bordetella virus BPP1: NP_958686.1; Salmonella phage epsilon15: NP_848215.1; Salmonella virus P22: YP_063716.1; Pseudomonas virus LUZ24: YP_001671935.1; Escherichia virus phiEco32: YP_001671756.1; Vibrio phage vB_ValP_IME271: ASR73870.1; Vibrio phage VPp1: AII27504.1; Escherichia phage vB_EcoP_G7C: YP_004782186.1; Brucella phage Pr: YP_007002083.1; Brucella phage Tb: AHB81300.1; Erwinia phage Ea9-2: YP_009007453.1; Vibrio phage VP3: AFH14435.1; Vibrio phage VP4: YP_249588.2; Vibrio phage N4: YP_003347932.1; Vibrio phage ICP3: YP_004251282.1; Burkholderia virus Bcep22: AAQ54988.1; Vibrio phage c1: AHN84658.1; Vibrio phage Vp670: APU00182.1; Vibrio phage phi-A318: YP_009110743.1; Vibrio phage AS51: AHC94068.1; Streptococcus phage Cp-1: NP_044821.1; Bacillus virus phi29: YP_002004536.1; Xylella phage Prado: YP_008859419.1; Staphylococcus virus 44AHJD: NP_817314.1; Klebsiella phage KP32: YP_003347548.1; Enterobacteria phage T7: NP_041997.1; Pseudomonas phage phiKMV: NP_877471.1; Acinetobacter phage Fri1: YP_009203047.1; Klebsiella phage KP34: YP_003347636.1.)

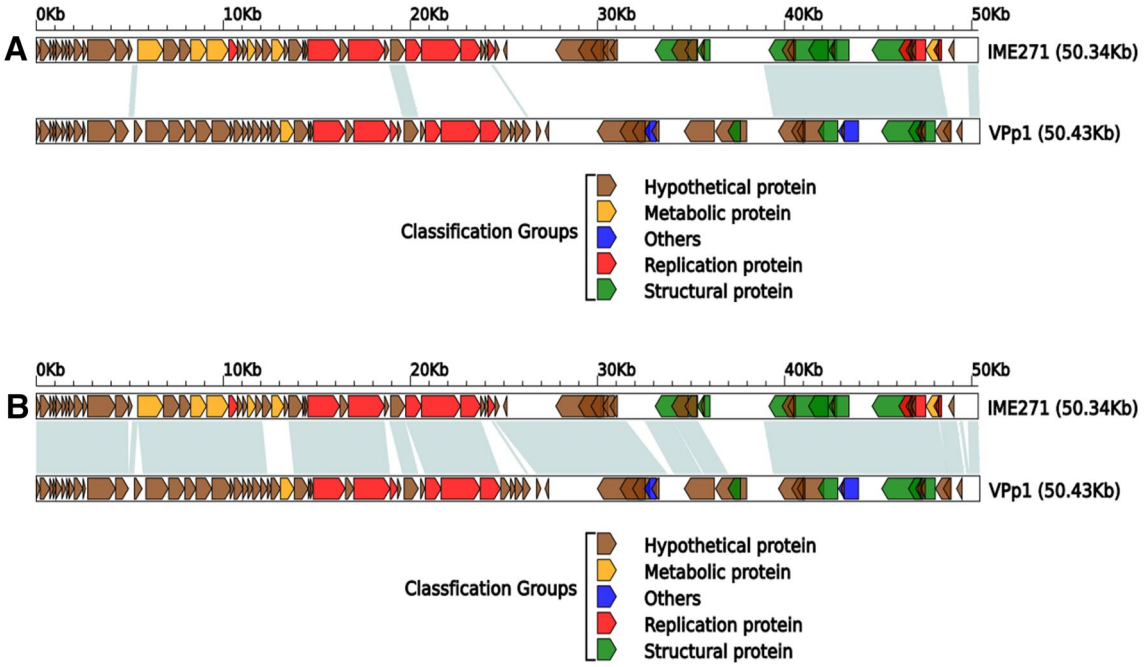

Fig. 4 Comparative analysis of vB_ValP_IME271 and VPp1. **a** Comparative analysis of these two phages was conducted using Easyfig, with nucleotide identity above 95%. **b** Comparative analysis of these two phages was conducted using Easyfig, with nucleotide identity above 90%. Well conserved segments are paired by shaded regions, and arrows indicate the direction of transcription for the predicted ORFs. Functional ORFs were classified into five groups: brown, hypothetical proteins; green: structural protein; blue: other proteins; red: replication proteins

is not known whether this phage carries potentially hazardous genes; therefore, the safety of vB_ValP_IME271 should be carefully evaluated in any future study before it is used as a therapy. It is expected that more *Vibrio* phages will be discovered in the future, and the information gained from them will help to provide more comprehensive information for phage therapy.

Nucleotide sequence accession number The nucleotide sequence of the phage vB_ValP_IME271 genome has been deposited in GenBank under Accession Number MF431726.

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Author contributions LZ and YT conceived and designed the experiments and critically evaluated the manuscript. FL and TM isolated and identified the phage and conducted the biological characterization experiments. ZX was responsible for the data and sequence analyses and wrote the manuscript. KF, SZ, and JL collected the clinical bacteria and carried out the experiments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research does not contain any studies with human participants or animals that were performed by any of the authors.

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