Isolation and characterization of the first phage infecting ecologically important marine bacteria *Erythrobacter*

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**Abstract**

**Background:** *Erythrobacter* comprises a widespread and ecologically significant genus of marine bacteria. However, no phage infecting *Erythrobacter* spp. has been reported to date. This study describes the isolation and characterization of phage vB_EliS-R6L from *Erythrobacter*.

**Methods:** Standard virus enrichment and double-layer agar methods were used to isolate and characterize the phage. Morphology was observed by transmission electron microscopy, and a one-step growth curve assay was performed. The phage genome was sequenced using the Illumina Miseq platform and annotated using standard bioinformatics tools. Phylogenetic analyses were performed based on the deduced amino acid sequences of terminase, endolysin, portal protein, and major capsid protein, and genome recruitment analysis was conducted using Jiulong River Estuary Virome, Pacific Ocean Virome and Global Ocean Survey databases.

**Results:** A novel phage, vB_EliS-R6L, from coastal waters of Xiamen, China, was isolated and found to infect the marine bacterium *Erythrobacter litoralis* DSM 8509. Morphological observation and genome analysis revealed that phage vB_EliS-R6L is a siphovirus with a 65.7-kb genome that encodes 108 putative gene products. The phage exhibits growth at a wide range of temperature and pH conditions. Genes encoding five methylase-related proteins were found in the genome, and recognition site predictions suggested its resistance to restriction-modification host systems. Genomic comparisons and phylogenetic analyses indicate that phage vB_EliS-R6L is distinct from other known phages. Metagenomic recruitment analysis revealed that vB_EliS-R6L-like phages are widespread in marine environments, with likely distribution in coastal waters.

**Conclusions:** Isolation of the first *Erythrobacter* phage (vB_EliS-R6L) will contribute to our understanding of host-phage interactions, the ecology of marine *Erythrobacter* and viral metagenome annotation efforts.

**Keywords:** *Erythrobacter*, Marine, Siphovirus, Complete genome sequence

**Background**

As ecologically significant marine bacteria, *Erythrobacter* species (Alphaproteobacteria) are frequently detected in and isolated from nutrient-rich coastal seawaters [1–5]. Moreover, these microorganisms are thought to comprise a major fraction of the marine phototrophs known as aerobic anoxygenic phototrophic bacteria (AAPBs), which play a significant role in the cycling of both organic and inorganic carbon in the ocean [2, 6–8]. To date, 19 *Erythrobacter* species have been reported, and genomic and metabolic studies have shown that members of this genus are metabolically versatile [5, 9–11]. The first marine *Erythrobacter* isolate was *E. longus* DSM 6997, which was also the first AAPB identified [1]. In 1994, *E. litoralis* DSM 8509, containing the carotenoids bacteriorubaxanthin and erythroxanthin sulfate, was isolated from a marine cyanobacterial mat [12]. In addition, previous studies have demonstrated the potential use of *Erythrobacter* species (e.g., *E. longus* and *E. citreus*) for bioremediation of alkane contamination [13]. These species show high levels of resistance to tellurite and accumulate metallic tellurium crystals (e.g., *E. longus*) [14].
epoxide hydrolase activity (e.g., *E. longus*) has also been reported [15].

Bacteriophages (viruses that infect bacteria) have important roles in the abundance, activity, and diversity of bacterial communities [16–18], and isolation and genomic characterization of phages greatly improves our understanding of the ecology and evolution of their hosts. For example, cyanophages (viruses that infect cyanobacteria) are active and abundant agents of mortality that directly affect the distribution and species composition of cyanobacteria in the aquatic environment [17, 19]. In addition, investigation of SAR11 viruses helped to show that the highly abundant distribution of these viruses is the result of adaptation to resource competition [20]. It has also been suggested that roseophages (viruses that infect *Roseobacter* species, another representative genus of Alphaproteobacteria) can quickly alter the growth and abundance of their host population by changing their infection strategy and can shunt bacterial secondary production into the environmental dissolved-carbon pool [e.g., 21, 22].

Isolation of novel phages can assist with both the annotation of unidentified functional genes and in the discovery of diverse and widespread viral assemblages in aquatic and marine environments through virome database query [20, 22, 23]. However, no phage infecting *Erythrobacter* has been reported to date, hindering an integrated understanding of the life cycle of these microbes in the ocean. In this study, we report the first isolation of a novel phage infecting *E. litoralis* DSM 8509.

**Methods**

**Bacterial strains and growth conditions**

All of the bacterial strains used in this study are listed in Table 1. *E. litoralis* DSM 8509 and other strains were cultivated at 30 °C in RO medium, an artificial seawater medium containing 1 g/L yeast extract, 1 g/L tryptone, and 1 g/L sodium acetate at pH 7.5 [24].

**Isolation of the phage**

Phage vB_EliS-R6L was isolated from seawater obtained in March 2014 off the coast of Xiamen, China (118°04’ E, 24°31’ N), using standard virus enrichment and double-layer agar methods. Briefly, *E. litoralis* DSM 8509 (100 mL) was co-cultured with a pre-filtered (0.22-μm membrane filter; Millipore, USA) seawater sample (20 mL) for 24 h at 30 °C. The culture was filtered again and serially diluted to determine phage activity using a double-layer agar method [25]. A single plaque was collected from the plate using a sterile pipette (Fisher, Canada) and then purified four successive times using the double-layer agar method. Following purification, stock cultures of the phage were prepared using sodium chloride-magnesium sulfate (SM) buffer (100 mM NaCl, 50 mM Tris, 10 mM MgSO4, and 0.01% gelatin, pH 7.5) supplemented with several drops of chloroform and stored at 4 °C and −80 °C.

**Transmission electron microscopy (TEM)**

For TEM analysis, 1 L of *E. litoralis* DSM 8509 culture (OD600 = 0.5) was inoculated with the phage at a multiplicity of infection of 10 and cultivated for 24 h at 30 °C. The culture was centrifuged at 6000×g for 10 min, and the upper aqueous phase was filtered through a 0.22-μm membrane and precipitated with 10% (w/v) dissolved polyethylene glycol 8000 (containing 1 M NaCl). After >8 h at 4 °C, the mixture was centrifuged at 10,000×g for 50 min at 4 °C, and the pellet was gently resuspended in 5 mL of SM buffer. The phages were then purified by CsCl gradient ultra-centrifugation (gradient-density: 1.5 g/mL, 200,000×g, 24 h, 4 °C; Optima L-100 XP Ultracentrifuge, Beckman Coulter). The purified phage particles were collected and dialyzed twice in SM buffer; 20 μL of suspension was added dropwise onto a copper grid and negatively stained with 2% aqueous uranyl acetate for 10 min. Transmission electron micrographs were obtained using a JEM-2100HC transmission electron microscope (JEOL, Japan) at an accelerating voltage of 120 kV. The phage size was calculated from at least 20 particles.

**Chloroform sensitivity**

To determine whether phage vB_EliS-R6L contains lipids, its sensitivity to chloroform was examined as described previously [26]. Briefly, 500 μL of the phage suspension (~107 plaque forming units (PFU)/mL) were mixed with 5 μL, 50 μL, or 500 μL of chloroform, vigorously shaken for 2 min, and then incubated at 30 °C for 30 min. The samples were immediately diluted and plated for phage titration using double-layer agar plates inoculated with *E. litoralis* DSM 8509.

**Host range analysis**

To investigate the host range of phage vB_EliS-R6L, plaque assays were performed on 27 marine bacterial strains, including 21 *Erythrobacter* strains, two *Citromicrobium* strains, and one each of the genera *Roseobacter*, *Dinoroseobacter*, *Lutibacterium*, and *Halomonas* (Table 1). The host range was determined by adding 5 μL of a diluted phage suspension (~103 PFU/mL) dropwise onto the surface of double-layer agar plates inoculated with the bacterial strain of interest. The plates were incubated at 30 °C for up to 7 days, and plaque formation was assessed repeatedly during this period. The efficiency of plating (EOP) of susceptible strains was quantified by calculating the ratio of the PFU obtained with each phage-susceptible strain to the PFU obtained with *E. litoralis* DSM 8509. All assays were carried out in triplicate.
| Strains | Best matched species (% Id of 16S rDNA) | Source and location | References | Susceptibility to phage vB_EliS-R6L | Efficiency of plaquing |
|---------|-----------------------------------------|---------------------|------------|-----------------------------------|------------------------|
| *Erythrobacter litoralis* DSM 8509* | *Erythrobacter litoralis* sp. CC-AMZ-30 L (97.12) | Surface sea water, Pacific Ocean | [12] | + | 100% |
| *Erythrobacter longus* DSM 6997* | *Erythrobacter longus* sp. M71_W20 (100.00) | Surface sea water, Taiwan strait, China | [1] | + | 94.74 ± 3.78% |
| *Erythrobacter* sp. JL 475 | *Erythrobacter* sp. JL 475 | Surface sea water, South China sea, China | [11] | - | - |
| *Erythrobacter* sp. JL 971 | *Erythrobacter* sp. JL 971 | Surface sea water, Taiwan strait, China | - | - | - |
| *Erythrobacter* sp. JL 1059 | *Erythrobacter* nanhaisediminis T30 (99.22) | Upper sea water (150 m), West Pacific Ocean | - | - | - |
| *Erythrobacter* sp. JL 1033 | *Erythrobacter* nanhaisediminis T30 (99.69) | Upper sea water (50 m), West Pacific Ocean | - | - | - |
| *Erythrobacter* sp. JL 1302 | *Erythrobacter* nanhaisediminis T30 (97.79) | Surface sea water, South China sea, China | - | - | - |
| *Erythrobacter* vulgaris TVG01-C004 (99.80) | *Erythrobacter* vulgaris TVG01-C004 (99.80) | Surface sea water, West Pacific Ocean | - | - | - |
| *Erythrobacter* vulgaris JL 1500 | *Erythrobacter* vulgaris 022 2–10 (99.22) | Changjiang Estuary, China | - | - | - |
| *Erythrobacter* vulgaris JL 1463 | *Erythrobacter* vulgaris JL 1463 | Surface sea water, Beibu Gulf, China | - | - | - |
| *Erythrobacter* flavus SW-46 (99.79) | *Erythrobacter* flavus SW-46 (99.79) | Surface sea water, Taiwan strait, China | - | - | - |
| *Erythrobacter* flavus SW-46 (99.25) | *Erythrobacter* flavus SW-46 (99.25) | Surface sea water, South China sea, China | - | - | - |
| *Erythrobacter* flavus BL16 (100.00) | *Erythrobacter* flavus BL16 (100.00) | Bottom sea water, South China sea, China | - | - | - |
| *Erythrobacter* flavus SW-46 (99.89) | *Erythrobacter* flavus SW-46 (99.89) | Surface sea water, South China sea, China | - | - | - |
| *Erythrobacter* flavus RE35F/1 (99.72) | *Erythrobacter* flavus RE35F/1 (99.72) | Surface sea water, Taiwan strait, China | - | - | - |
| *Erythrobacter* flavus RE35F/1 (99.01) | *Erythrobacter* flavus RE35F/1 (99.01) | Surface sea water, South China sea, China | - | - | - |
| *Erythrobacter* citreus RE35F/1 (99.66) | *Erythrobacter* citreus RE35F/1 (99.66) | Surface sea water, Taiwan strait, China | - | - | - |
| *Roseobacter denitrificans* OCH114 DSM 7001* | *Roseobacter denitrificans* OCH114 DSM 7001* | Seaweed, Japan | [52] | - | - |
| *Dinoroseobacter shibae* DFL12* | *Dinoroseobacter shibae* DFL12* | Cells of Prorocentrum lima | [53] | - | - |
| *Citromicrobium bathyomarinum* JL 354 | *Citromicrobium bathyomarinum* JL 354 | Surface sea water, South China sea, China | - | - | - |
| *Citromicrobium* sp. (100.00) | *Citromicrobium* sp. (100.00) | Upper sea water (50 m), South China sea, China | - | - | - |
| *Lutibacterium* sp. (100.00) | *Lutibacterium* sp. (100.00) | Surface sea water, Atlantic Ocean | - | - | - |
| *Halomonas* sp. (100.00) | *Halomonas* sp. (100.00) | Surface sea water, Pacific Ocean | - | - | - |

*strains were purchased from DSMZ (the German Resource Center for Biological Material), Germany. +, cell lysis; –, no effect.
One-step growth assays

One-step growth curve experiments were performed as previously described [25, 27]. Briefly, mid-exponential phase *E. litoralis* DSM 8509 (optical density at 600 nm = 0.3–0.5, 100 mL) was inoculated with phage at a multiplicity of infection of 0.01 and allowed to adsorb for 10 min at 30 °C. The mixture was then centrifuged at 6000×g for 10 min to remove non-absorbed phage in the supernatant; the pelleted cells were resuspended in 100 mL of RO medium, followed by incubation at 30 °C. Two sets of duplicate samples were removed at 20-min intervals for 6 h, and chloroform (1% final concentration) was added to the second set to release the intracellular phage. The two samples were then diluted and immediately plated for phage titration using the double-layer agar method. Another set of cultures without phage in untreated cultures were removed at the 20-min intervals for 6 h and at 1-h intervals for the next 4 h. The PFU of each sample was calculated by counting the plaques on the bacterial lawn. The assay was performed in triplicate.

Thermal/pH stability

To investigate the thermal stability of the phage, 1 mL of phage vB_EliS-R6L (~10^7 PFU/mL) with SM buffer was incubated for 2 h at 30 °C, 40 °C, 42.5 °C, 45 °C, 50 °C, 60 °C, 70 °C, 75 °C, or 80 °C, after which the phage suspensions were immediately cooled to 4 °C for activity estimation. To evaluate the stability of the phage at different pH levels, RO medium was adjusted to pH 1–14 with sterile 5 M HCl or NaOH solution and then filtered through a 0.22-μm membrane filter (Millipore, USA). Additionally, 1 mL of a phage suspension (~10^7 PFU/mL) prefiltered through a 0.22-μm membrane filter was incubated at 30 °C for 24 h in 9 mL of RO medium of different pHs. Phage activity was determined using the double-layer agar method with RO medium (pH 7.5) at 30 °C and assessed by calculating changes in PFU following exposure to the different temperatures and pH levels. All assays were performed in triplicate.

Effects of temperature and pH on infection

To investigate the effect of temperature on phage infection, 5 μL of a phage suspension (~10^9 PFU/mL) was added dropwise onto double-layer agar plates inoculated with host cells at different pH values. The plates were incubated at 30 °C for up to 7 days. All assays were performed in triplicate.

Lysogenic/lytic assays

To investigate whether the phage can integrate onto the genome of its host, 10 μL of a phage suspension (~10^9 PFU/mL) was added dropwise onto double-layer agar plates inoculated with *E. litoralis* DSM 8509; the center portion within the plaques was carefully pipetted out and inoculated onto a new plate. After two rounds of isolation and purification, 40 randomly selected bacterial colonies were chosen for colony polymerase chain reaction (PCR) using two pairs of primers, designed according to phage genome annotation, targeting ORF 91 (Major capsid protein) (forward primer 5′–GCTGACCACCAAGCGATGA – 3′, reverse primer 5′– CGGAACGAGGCTATCCAC – 3′, 521 bp) and ORF 100 (Terminase) (forward primer 5′–TCTGTGGCAGGCTTGGG – 3′, reverse primer 5′–GGTCTGGTCC AGTCTTTCCG – 3′, 549 bp).

Phage DNA extraction, sequencing, and genomic analysis

Using the same sample preparation utilized for TEM analysis, 1 mL of a phage suspension was purified by CsCl density-gradient centrifugation, followed by dialysis. To remove free DNA and RNA, the sample was then digested at 37 °C for 1 h with DNase I and RNase A (Takara) at final concentrations of 1 μg/mL. The solution was incubated with proteinase K and sodium dodecyl sulfate at final concentrations of 100 μg/mL and 1% (w/v), respectively, at 55 °C for 2 h. After incubation, the solution was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1), after which the solutions were precipitated with sodium acetate and precooled ethanol at final concentrations of 1/10 and 1/1 (v/v), respectively. After overnight incubation at −20 °C, DNA was collected by centrifugation and successively washed twice with precooled 70% and 100% ethanol. The genomic DNA of vB_EliS-R6L was sequenced using the Illumina Miseq platform to generate 2 × 251 bp paired-end reads. The reads were assembled using CLC Genomics Workbench software (18,777 × coverage).

Genomic and bioinformatic analyses

The GeneMarkS online server (http://exon.gatech.edu/GeneMark/genemarks.cgi), Glimmer 3.0 (http://cgb.jhu.edu/software/glimmer/index.shtml), and the ORF Finder online server (https://www.ncbi.nlm.nih.gov/orffinder/) were used to identify putative open reading frames (ORFs). Genes were annotated using BLAST searches against the NCBI non-redundant (nr) protein database,
with a cut-off of E-value $\leq 10^{-5}$. A temperate and/or lytic lifestyle was predicted using the phage classification toolset (PHACTS) online prediction program (http://www.phantome.org/PHACTS/index.php). Methylation ORFs were searched using the REBASE online program (http://rebase.neb.com/rebase/rebase.html).

The amino acid sequences of endolysin protein (ORF 74), major capsid protein (ORF 91), portal protein (ORF 98), and terminase (ORF 100) from phage vB_EliS-R6L were used to construct neighbor-joining phylogenetic trees with MEGA 6.06 and 200 bootstrap replications. For use in the phylogenetic analysis, the amino acid sequences of these four proteins from closely related phages were retrieved from GenBank.

**Genome recruitment**

To explore the geographic distribution of vB_EliS-R6L-like phages, the amino acid sequences of the phage ORFs were employed as queries to search against metagenomic databases of the Jiulong River Estuary (JRE), Xiamen, China [28], the Pacific Ocean Virome (POV) and Global Ocean Survey (GOS) (http://data.imicrobe.us/) using BLASTn at a cut-off of E-value $\leq 10^{-5}$, an alignment value $\geq 30$ and a score value $\geq 40$. The count abundance of each read was normalized by dividing by the number of total reads in the database and the size of the gene product [20].

**Nucleotide sequence accession number**

The genome sequence of phage vB_EliS-R6L was deposited in the GenBank database under accession number KY006853.

**Results and discussion**

**Phage isolation and basic characterization**

To the best of our knowledge, vB_EliS-R6L is the first phage isolated from the ecologically important marine bacteria of the genus *Erythrobacter*. vB_EliS-R6L forms small, clear, round (1–4 mm diameter) plaques on a bacterial lawn (Fig. 1). After treatment with different concentrations of chloroform (i.e., 1%, 10%, and 100% (v/v)), the phage showed survival rates of 94.7 ± 4.9%, 83.1 ± 2.5%, and 81.9 ± 1.9%, respectively, indicating that vB_EliS-R6L may not sensitive to chloroform or contain lipids. TEM micrographs revealed that it belongs to the siphovirus family, with an isosahedral capsid 75.9 ± 2.2 nm in diameter and a characteristically long tail of 165.6 ± 2.3 nm (Fig. 1).

Of all the strains tested, phage vB_EliS-R6L could only infect *E. litoralis* DSM 8509 and *E. longus* DSM 6997, the only strains for each species that could be obtained from public culture collections. Of the commonly isolated three-tailed phage families (Myoviridae, Siphoviridae, and Podoviridae), Myoviridae phages have a broader host range than species of the other two families. Therefore, it was not unexpected that a narrow host range was observed for phage vB_EliS-R6L. Based on whole-genome comparison, Zheng et al. (2016) reported that *Erythrobacter* strains cluster into three groups, with strains DSM 8509, DSM 6997, and JL 475 belonging to the same group. These three strains share high 16S rRNA gene identity (> 97%) but can be discriminated by average nucleotide identity analysis [11]. Integrative and conjugative element analysis showed that DSM 8509 and DSM 6997 cluster closely together and away from JL 475, suggesting asynchronous evolution. This may account for the ability of phage vB_EliS-R6L to infect DSM 8509 and 6997 but not JL 475. In addition, previous studies have suggested that the number of tRNAs can be positively correlated with host range due to compensation for different codon usage patterns in host bacteria [29]. No tRNAs were identified in the phage vB_EliS-R6L genome using tRNAscan-SE (1.3.1) software [30], which may also account for its relatively narrow host range. In lysogenic/lytic assays, 17.5% (7/40) of bacterial isolates from the center portion of plaques showed positive PCR amplification using primers specific for phage ORFs. This finding suggests that vB_EliS-R6L may integrate into its host cell and possibly enter into a lysogenic life cycle, which is consistent with our bioinformatic analysis (see below).

According to one-step growth curve experiments, the eclipse and latent periods of phage infection occurred at 2 h 40 min and 3 h post-infection, respectively (Fig. 2). The burst size was ~86 PFU/cell, similar to the latent period and burst size of most phages infecting *Roseobacter* species, ranging from <1–6 h and 27–1500 PFU/cell, respectively [23, 31–33]. Stability assessment showed that over 60% of vB_EliS-R6L phage remained active at temperatures up to 50 °C (2 h treatment) and that <1% remained active at temperatures >70 °C (Fig. 3). In addition, the phage survival rate was greater than 77%
after 24 h at pH 6, 7, or 8 (Fig. 3). Although vB_EliS-R6L retained some activity after 24 h at pH 3 (39%) and pH 12 (10%), activity was lost below pH 2 or above pH 13. An infection condition test showed that phage vB_EliS-R6L could infect *E. litoralis* DSM 8509 and form clear plaques on plates within 2 days at 25 °C ~ 35 °C. Visible plaques appeared on plates after 4 days at 15 °C and 20 °C, whereas no clear plaques were visible at 40 °C after 7 days of incubation. In addition, plaques were observed in the infection test within a pH range of 7–10. These data showed phage vB_EliS-R6L particles to be stable, with broad temperature and pH tolerance compared to most isolated phages [34], characteristics that might offer more survival opportunities in the diverse marine environment. However, phage vB_EliS-R6L was only able to successfully proliferate within a relatively narrow range of conditions (i.e., < 40 °C, pH 7–10). Unsuccessful infection might be a consequence of thermal/chemical alterations to the phage structure or host receptors [35, 36], and further investigation is needed.

**Genomic analysis of phage vB_EliS-R6L**

The complete dsDNA genome of phage vB_EliS-R6L is 65,675 bp in size (GenBank accession no. KY006853). The overall G + C content is 66.5%, similar to that of its host (i.e., 65.2%, GenBank accession no. NZ_CP017057). A total of 108 ORFs were identified (Table 2), and identity of the predicted coding sequences with sequences available in GenBank is low (26–77% at the amino acid level). Homologous sequences in the NCBI non-redundant protein database were found for 58 gene products; however, only 29 had predicted functions (Table 2), 19 of which have been assigned to known functional domain categories. In total, 27 ORFs are homologous with previously identified bacteriophage genes, and 15 are homologs of proteins from siphophage-infecting Alphaproteobacteria.
| Gene | Strand | Start (bp) | Stop (bp) | Residue length (nn) | Residue length (aa) | Putative function/feature | Best matched evidence or organism | Homolog Accession Num. | % Id | BlastP E-Value |
|------|--------|------------|-----------|--------------------|--------------------|--------------------------|----------------------------------|--------------------------|------|----------------|
| 1    | -      | <3         | 2423      | 2421               | 830                | DNA modification methylase | uncultured Mediterranean phage uvMED | BAQ92410                 | 55   | 2.00E-47      |
| 2    | -      | 2420       | 2875      | 456                | 151                | hypothetical protein (DNA polymerase III beta clamp) | Methylobacterium sp. ARG-1 (Caulobacter phage Sansa) | WP_050734237 (AKU43506) | 49   | 7.00E-11 (3.00E-04) |
| 3    | -      | 2883       | 3239      | 357                | 118                | hypothetical protein | None | n/a | n/a |
| 4    | -      | 3286       | 3456      | 171                | 56                 | hypothetical protein | None | n/a | n/a |
| 5    | -      | 3453       | 3614      | 162                | 53                 | hypothetical protein | None | n/a | n/a |
| 6    | -      | 3601       | 3813      | 213                | 70                 | hypothetical protein | None | n/a | n/a |
| 7    | -      | 3810       | 4853      | 1044               | 347                | phosphoadenosine phosphosulfate reductase | Sphingomonas sp. L128 | WP_008827527 | 49 | 3.00E-85 |
| 8    | -      | 4835       | 5023      | 189                | 62                 | hypothetical protein | None | n/a | n/a |
| 9    | -      | 5020       | 5628      | 609                | 202                | molecular chaperone | Caulobacter phage Sansa | AKU43478 | 41 | 5.00E-42 |
| 10   | -      | 5674       | 6315      | 642                | 213                | hypothetical protein | Pseudomonas oryzihabitans | WP_044342705 | 55 | 2.00E-63 |
| 11   | -      | 6322       | 6558      | 237                | 78                 | hypothetical protein | None | n/a | n/a |
| 12   | -      | 6537       | 6791      | 255                | 84                 | hypothetical protein | Sphingomonas sp. Ant H11 | WP_052192475 | 52 | 3.00E-23 |
| 13   | -      | 6791       | 7174      | 384                | 127                | hypothetical protein | uncultured Mediterranean phage uvMED (Caulobacter phage Sansa) | BAR28076 (AKU43468) | 39 | 5.00E-08 (7.00E-04) |
| 14   | -      | 7171       | 7473      | 303                | 100                | hypothetical protein | None | n/a | n/a |
| 15   | -      | 7473       | 7904      | 432                | 143                | hypothetical protein | None | n/a | n/a |
| 16   | -      | 7901       | 8050      | 150                | 49                 | hypothetical protein | None | n/a | n/a |
| 17   | -      | 8047       | 8397      | 351                | 116                | hypothetical protein | None | n/a | n/a |
| 18   | -      | 8394       | 8933      | 540                | 179                | hypothetical protein | None | n/a | n/a |
| 19   | -      | 8933       | 9061      | 129                | 42                 | hypothetical protein | None | n/a | n/a |
| 20   | -      | 9061       | 9198      | 138                | 45                 | hypothetical protein | None | n/a | n/a |
| 21   | -      | 9195       | 9422      | 228                | 75                 | hypothetical protein | None | n/a | n/a |
| 22   | -      | 9419       | 9679      | 261                | 86                 | hypothetical protein | Pseudomonas phage P106B-62 | YP_009005988 | 47 | 2.00E-08 |
| 23   | -      | 9733       | 10,005    | 273                | 90                 | hypothetical protein | None | n/a | n/a |
| 24   | -      | 10,005     | 10,118    | 114                | 37                 | hypothetical protein | None (Caulobacter phage Sansa) | n/a (AKU43430) | n/a | n/a |
| 25   | -      | 10,115     | 10,603    | 489                | 162                | hypothetical protein | None | n/a | n/a |
| 26   | -      | 10,596     | 11,546    | 951                | 316                | hypothetical protein | Rhizobium tropici | WP_052227599 | 45 | 1.10E-02 |
| 27   | -      | 11,697     | 12,263    | 567                | 188                | hypothetical protein | Sphingomonas sp. Y57 | WP_053000396 | 41 | 3.00E-31 |
| 28   | -      | 12,278     | 12,880    | 603                | 200                | hypothetical protein | Navosphingobium sp. ST904 | WP_054436273 | 39 | 3.00E-23 |
| 29   | -      | 12,867     | 13,688    | 822                | 273                | hypothetical protein | Erythrobacter sp. SG61-1 L | WP_054529722 | 40 | 3.00E-10 |
| 30   | -      | 13,961     | 14,176    | 216                | 71                 | hypothetical protein | None | n/a | n/a |
Table 2  *Erythrobacter litoralis* DSM 8509 phage vB_EliS-R6L genome annotations (KY006853) (Continued)

|   | Start | End | Length | E-value | Function | Species | Accession | Gene | Start | End | Length | E-value | Function | Species | Accession | Gene | Start | End | Length | E-value |
|---|-------|-----|--------|---------|----------|---------|-----------|------|-------|-----|--------|---------|----------|---------|-----------|------|-------|-----|--------|---------|
| 31 | 14,173 | 14,859 | 687 | 228 | hypothetical protein | *Lactobacillus* phage LL-H | YP_0001285924 | 43 | 1.00E-18 |
| 32 | 14,856 | 15,137 | 282 | 93 | acyl carrier protein | *Ruminococcus albus* | WP_037276568 | 44 | 6.00E-13 |
| 33 | 15,195 | 15,818 | 624 | 207 | hypothetical protein | *Sphingomonas wittichii* | WP_016745765 | 29 | 6.00E-10 |
| 34 | 15,808 | 16,488 | 681 | 226 | methyltransferase | *Caulobacter phage Sansa* | AKU43482 | 31 | 2.00E-08 |
| 35 | 16,488 | 16,703 | 216 | 71 | hypothetical protein | None | n/a | n/a | n/a |
| 36 | 16,834 | 17,106 | 273 | 90 | hypothetical protein | None | n/a | n/a | n/a |
| 37 | 17,106 | 17,384 | 279 | 92 | hypothetical protein | None | n/a | n/a | n/a |
| 38 | 17,381 | 17,662 | 282 | 93 | hypothetical protein | None | n/a | n/a | n/a |
| 39 | 17,664 | 17,909 | 246 | 81 | hypothetical protein | None | n/a | n/a | n/a |
| 40 | 17,986 | 18,774 | 789 | 262 | type I restriction-modification system methyltransferase subunit-like protein | *Methylobacterium nodulans* ORS 2060 | YP_009126070 | 41 | 2.71E-44 |
| 41 | 18,774 | 20,180 | 1407 | 468 | nucleoside triphosphate hydrolase | *Caulobacter phage Sansa* | AKU43472 | 37 | 1.00E-59 |
| 42 | 20,177 | 20,440 | 264 | 87 | hypothetical protein | *Sphingomonas sp. BHC-A* | WP_025772276 | 51 | 1.00E-13 |
| 43 | 20,437 | 20,730 | 294 | 97 | hypothetical protein | None | n/a | n/a | n/a |
| 44 | 20,723 | 20,983 | 261 | 86 | hypothetical protein | None | n/a | n/a | n/a |
| 45 | 20,980 | 22,629 | 1650 | 549 | nucleic acid-binding protein | *Caulobacter phage Sansa* | AKU43470 | 30 | 2.00E-12 |
| 46 | 22,626 | 23,351 | 726 | 241 | exonuclease | *Sphingobium baderi LL03 (Caulobacter phage Sansa)* | KMS62764 (AKU43467) | 46 | 8.00E-64 (1.00E-22) |
| 47 | 23,341 | 24,513 | 1173 | 390 | ERF family protein | *Dunaliella viridis virus S12* | YP_009021005 | 31 | 1.00E-19 |
| 48 | 24,513 | 24,677 | 165 | 54 | hypothetical protein | None | n/a | n/a | n/a |
| 49 | 24,677 | 25,180 | 504 | 167 | single-stranded DNA-binding protein | *Citromicrobium (Caulobacter phage Sansa)* | WP_010236565 (AKU43479) | 63 | 2.00E-56 (1.00E-52) |
| 50 | 25,192 | 26,049 | 858 | 285 | phage Gp37Gp68 (ssDNA-annealing protein) | *Sphingomonas sp. Y57 (Caulobacter phage Sansa)* | WP_047169428 (AKU43469) | 52 | 3.00E-89 (9.00E-05) |
| 51 | 26,046 | 26,573 | 528 | 175 | hypothetical protein | None | n/a | n/a | n/a |
| 52 | 26,598 | 27,239 | 642 | 213 | hypothetical protein | None (Caulobacter phage Sansa) | n/a (AKU43520) | n/a | 3.00E-04 |
| 53 | 27,232 | 27,639 | 408 | 135 | hypothetical protein | *Sphingobium chungbukense* | WP_046763480 | 43 | 7.00E-15 |
| 54 | 27,873 | 28,238 | 366 | 121 | hypothetical protein | *Pseudomonas aeruginosa* | WP_052157666 | 48 | 2.00E-11 |
| 55 | 28,309 | 28,692 | 384 | 127 | cytosine-specific methyltransferase | *Ralstonia solanacearum GM11000* | NP_518991 | 48 | 7.98E-23 |
| 56 | 28,730 | 28,918 | 189 | 62 | hypothetical protein | None | n/a | n/a | n/a |
| 57 | 28,918 | 29,508 | 591 | 196 | hypothetical protein | None | n/a | n/a | n/a |
| 58 | 29,612 | 30,007 | 396 | 131 | hypothetical protein | None | n/a | n/a | n/a |
| 59 | 30,092 | 30,574 | 483 | 196 | hypothetical protein | None | n/a | n/a | n/a |
| 60 | 30,574 | 30,828 | 255 | 84 | hypothetical protein | None | n/a | n/a | n/a |
| 61 | 30,903 | 31,304 | 402 | 133 | MucR family transcriptional regulator | *Methylobacterium nodulans* | WP_012631401 | 55 | 3.00E-33 |
Table 2 *Erythrobacter litoralis* DSM 8509 phage vB_EliS-R6L genome annotations (KY006853) (Continued)

| Start | Stop  | Length | Start | Stop  | Length | Start | Stop  | Length | Start | Stop  | Length | Start | Stop  | Length | Start | Stop  | Length |
|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|
| 62 +  | 31,301| 31,687 | 387   | 128   | hypothetical protein | None  | n/a   | n/a    | n/a |
| 63 +  | 31,684| 31,902 | 219   | 72    | hypothetical protein | None  | n/a   | n/a    | n/a |
| 64 +  | 31,893| 32,033 | 141   | 46    | hypothetical protein | None  | n/a   | n/a    | n/a |
| 65 +  | 32,020| 32,235 | 216   | 71    | hypothetical protein | None  | n/a   | n/a    | n/a |
| 66 +  | 32,235| 32,468 | 234   | 77    | hypothetical protein | None  | n/a   | n/a    | n/a |
| 67 +  | 32,465| 33,223 | 759   | 252   | DNA methylase | Mycobacterium phage Llama | AIMS1011 | 56 | 2.00E-51 |
| 68 +  | 33,259| 33,612 | 354   | 117   | hypothetical protein | Vibrio phage VvAW1 | YP_007518376 | 44 | 2.00E-20 |
| 69 +  | 33,662| 33,958 | 297   | 98    | hypothetical protein | Burkholderia vietnamiensis | WP_011875349 | 34 | 1.00E-05 |
| 70 +  | 33,955| 34,455 | 501   | 166   | hypothetical protein | Novosphingobium sp. KN65.2 | CDO34010 | 41 | 4.00E-22 |
| 71 -  | 34,717| 34,962 | 246   | 81    | hypothetical protein | Sphingomonas sanxanigenens | WP_025293719 | 52 | 5.00E-22 |
| 72 -  | 35,015| 35,344 | 330   | 109   | hypothetical protein | Sphingomonas sanxanigenens | WP_025293718 | 55 | 1.00E-08 |
| 73 -  | 35,316| 35,633 | 318   | 105   | hypothetical protein | None  | n/a   | n/a    | n/a |
| 74 -  | 35,630| 36,505 | 876   | 291   | endolysin | Caulobacter phage Sansa | AKU43454 | 50 | 2.00E-39 |
| 75 -  | 36,552| 36,776 | 225   | 74    | hypothetical protein | Sphingomonas sp. ATCC 3155S | WP_019371220 | 68 | 2.00E-12 |
| 76 -  | 36,935| 37,528 | 594   | 197   | hypothetical protein | Sphingomonas sp. ATCC 3155s | WP_019371221 | 44 | 2.00E-34 |
| 77 -  | 37,589| 39,856 | 2268  | 755   | D-alanyl-D-alanine carboxypeptidase | Methyloceanibacter caenitepidi | BAQ15659 | 33 | 2.00E-25 |
| 78 -  | 39,853| 40,308 | 456   | 151   | hypothetical protein | Delftia sp. RIT313 | WP_052155377 | 54 | 2.00E-27 |
| 79 -  | 40,309| 43,059 | 2751  | 916   | virion structural protein | Pseudomonas phage PaMx28 | ALH23633 | 44 | 0.00E + 00 |
| 80 -  | 43,088| 43,282 | 195   | 64    | tail assembly protein | Burkholderia phage AH2 | YP_006561132 | 53 | 1.00E-15 |
| 81 -  | 43,279| 43,509 | 231   | 76    | virion structural protein | Pseudomonas phage PaMx25 | ALH23804 | 77 | 5.00E-17 |
| 82 -  | 43,509| 44,288 | 780   | 259   | virion structural protein | Pseudomonas phage PaMx28 | ALH23630 | 48 | 2.00E-72 |
| 83 -  | 44,285| 45,874 | 1590  | 529   | tail assembly structural protein | Pseudomonas phage MP1412 | WP_006561079 | 35 | 1.00E-55 |
| 84 -  | 45,871| 49,170 | 3300  | 1099  | tail tape-measure protein | Paracoccus phage vB_PmaS_IMEP1 | YP_009126438 | 51 | 3.00E-41 |
| 85 -  | 49,462| 49,974 | 513   | 170   | hypothetical protein | Roseobacter phage RDJL Phi 2 | AKQ75858 | 26 | 8.00E-06 |
| 86 -  | 50,038| 51,579 | 1542  | 513   | major capsid protein | Roseobacter phage RDJL Phi 1 | YP_004421846 | 46 | 3.00E-13 |
| 87 -  | 51,592| 52,032 | 441   | 146   | phage structural protein | Roseobacter phage RDJL Phi 1 | YP_004421845 | 30 | 9.05E-13 |
| 88 -  | 52,029| 52,526 | 498   | 165   | virion structural protein | Pseudomonas phage PaMx25 | ALH23810 | 34 | 6.00E-06 |
| 89 -  | 52,530| 53,045 | 516   | 171   | hypothetical protein | None  | n/a   | n/a    | n/a |
| 90 -  | 53,136| 54,083 | 948   | 315   | hypothetical protein | Caulobacter phageSansa | AKU43432 | 61 | 9.00E-06 |
| 91 -  | 54,105| 55,115 | 1011  | 336   | major capsid protein E | Pseudomonas phage KPP23 (Caulobacter phage Sansa) | BAO53114 (AKU43431) | 32 | 8.00E-39 (9.00E-12) |
| 92 -  | 55,201| 55,578 | 378   | 125   | hypothetical protein | None  | n/a   | n/a    | n/a |
Overall, as suggested by the low degree of coverage (< 3%) of the entire genome sequence identified by BLASTn analysis, the vB_EliS-R6L genome is largely unique compared with other published phage genomes.

Eight genes were found to encode proteins related to DNA metabolism. In addition to DNA modification methylase (ORF 1) and DNA methylase (ORF 67), phage vB_EliS-R6L encodes another three methylase proteins, including a methyltransferase (ORF 34), a type I restriction-modification (R-M) system methyltransferase subunit-like protein (ORF 40), and a cytosine-specific methyltransferase (ORF 55). The identities range from 31 to 56% (46% on average). Four of the five ORFs are predicted to contain a single domain, including a CcrM-like domain (ORF 1, with recognition site GANTC), two SAM methyltransferase domains (COMT-like) (ORF 34 and 40, a versatile enzyme with various target molecules), and a Dam subfamily domain (ORF 67, with a recognition site of GATC). Methyltransferases are ubiquitous in prokaryotic genomes, and these enzymes are often associated with a cognate restriction endonuclease, forming an R-M system that protects bacterial cells from invasion by foreign DNA such as phages. Approximately 20% of annotated phage genomes encode methylases, and it is proposed that they may help the phage overcome R-M and other phage-targeted resistance systems in the host and prolong the effectiveness of infection [37]. As predicted by REBESE software, one R-M pair was recognized in the genome of E. litoralis DSM 8509 (with the recognition site CCGGAG), and five pairs were found for E. longus DSM 6997 (two of which have recognition sites GGCGCC and CGATCG; the other three have no recognition sites). Those R-M recognition sites indicate 37 potential cleavage sites (23 for CCGGAG, 14 for CGATCG) in the genome of phage vB_EliS-R6L. The predicted recognition site GATC of ORF 67 in the phage genome agrees with the R-M sites of DSM 6997, demonstrating the potential to overcome the host R-M system. Previous studies also found that phage T4 encodes a DAM methylase that targets GATC sites, protecting the phage DNA from an R-M system that recognizes this sequence [38]. Based on REBASE searches, 1051 homologs matched with the five methylase proteins, suggesting that R6L-like methylases are widespread, which may enhance infectivity and evasion of the host R-M system. Phage vB_EliS-R6L may represent a good model for exploitation of phage methylases and marine host-phage interactions. Moreover, Dziewit et al. (2014) suggested that methylases may account for differences in the methylation state and

| Table 2 | Erythrobacter litoralis DSM 8509 phage vB_EliS-R6L genome annotations (KY006853) (Continued) |
|---------|----------------------------------------------------------------------------------|
| 93 -    | 55,624 56,901 1278 425 hypothetical protein                                    | Roseobacter phage RDJL Phi 2                                      |
| 94 -    | 56,891 57,370 480 159 hypothetical protein                                    | Roseobacter phage RDJL Phi 2                                      |
| 95 -    | 57,494 58,489 996 331 head morphogenesis protein                              | Roseobacter phage RDJL Phi 2 (Caulobacter phage Sansa)           |
| 96 -    | 58,494 58,892 399 132 hypothetical protein                                    | Brucella abortus LMN1 KFH118426                                   |
| 97 -    | 58,892 59,431 540 179 hypothetical protein (tail protein)                    | Roseobacter phage RDJL Phi 2 (Caulobacter phage Sansa)           |
| 98 -    | 59,431 60,993 1563 520 portal protein                                        | Caulobacter phage Sansa                                          |
| 99 -    | 61,152 61,415 264 87 hypothetical protein                                    | None n/a n/a n/a                                               |
| 100 -   | 61,878 63,512 1635 544 terminase                                              | Agrobacterium rhizogenes WP_051696780                            |
| 101 -   | 63,493 64,023 531 176 hypothetical protein                                    | Nitratireductor basaltis WP_051913838                            |
| 102 +   | 64,145 64,327 183 60 hypothetical protein                                    | None n/a n/a n/a                                               |
| 103 +   | 64,371 64,556 186 61 hypothetical protein                                    | None n/a n/a n/a                                               |
| 104 +   | 64,533 64,687 135 44 hypothetical protein                                    | None n/a n/a n/a                                               |
| 105 +   | 64,747 65,037 291 96 hypothetical protein                                    | None n/a n/a n/a                                               |
| 106 +   | 65,037 65,204 168 55 hypothetical protein                                    | None n/a n/a n/a                                               |
| 107 +   | 65,233 65,520 288 95 hypothetical protein                                    | None n/a n/a n/a                                               |
| 108 +   | 65,532 65,675 144 47 hypothetical protein                                    | None n/a n/a n/a                                               |
induce host transcriptional changes that are essential for the phage life cycle [39].

Twelve ORFs are predicted to encode proteins involved in the structure and assembly of virions, nine of which are homologous to genes from Pseudomonas (Gammaproteobacteria) and/or Roseobacter isometric siphophages [21, 40, 41]. A further four conjunctive ORFs with unknown functions also exhibit homology to these phage types. This is consistent with the results of the phylogenetic trees generated using major capsid protein and portal protein amino acid sequences (Fig. 4). However, it is noteworthy that except for these 13 ORFs, no other ORFs of vB_EliS-R6L show a high degree of homology to Pseudomonas or Roseobacter isometric phage sequences. It therefore appears that genes associated with the structural architecture of phage vB_EliS-R6L are relatively conserved and may have evolved independently from other genes in the genome. Moreover, the low protein identity predicted between phage vB_EliS-R6L and those homologies (26–77%, 41% on average), as well as clearly distant phylogenetic relationships (Fig. 4), suggest that phage vB_EliS-R6L exchanged genetic material with those closely related phages prior to a distinct evolutionary path.

One putative endolysin gene (ORF 74) and one molecular chaperone (ORF 9) were identified in the genome of

![Phylogenetic relationships of four genes of vB_EliS-R6L-like phages. The neighbor-joining trees were based on the ClustalW alignment of amino acid sequences by MEGA 6.06. The bootstrap values were based on 200 replicates. (a) terminase; (b) endolysin; (c) portal protein; (d) major capsid protein.](image-url)
vB_EliS-R6L, sharing 50% and 41% amino acid identity, respectively, with the corresponding proteins of the Caulobacter phage Sansa [42]. Most tailed phages achieve lysis via consecutive use of two essential proteins, endolysin and holin (which control the length of the infective cycle). Endolysins are phage-encoded enzymes that degrade bacterial peptidoglycan. ORF 74 is predicted to contain one domain: a 176-aa region near the C-terminus that shows homology to proteins of the lysozyme-like superfamily. Although Caulobacter phage Sansa contains a lysis cassette (a holin/anti-holin pair and an endolysin) [42], none of the ORFs identified in phage vB_EliS-R6L exhibit homology to holin proteins. This may be the result of the limited number of holin protein sequences in databases [43, 44]. In addition, ORF 9 is predicted to contain one 49-aa domain homologous to chaperone J, which assists in translation.

Three ORFs are predicted to code for an acyl carrier protein (ORF 32), a nucleoside triphosphate hydrolase (ORF 41), and a phosphoadenosine phosphosulfate reductase (ORF 7). The acyl carrier protein in bacteria is responsible for fatty acid biosynthesis, requiring 4'-phosphopantetheine as a covalently attached cofactor. Acyl carrier protein homologs have also been identified in several other phages [45], though their function remains unclear. ORF 41 of phage vB_EliS-R6L is predicted to include a 292-aa P-loop domain of nucleoside triphosphate hydrolases, which hydrolyze the beta-gamma phosphate bond of a bound nucleoside triphosphate, providing energy for viral metabolism. ORF 7 shows 49% identity to phosphoadenosine phosphosulfate reductases, which have been identified in phages such as Lactobacillus phage AQ113 (GenBank accession no. HE956704) [46], Mycobacterium phage Baka (GenBank accession no. JF937090) [47], and Pseudoalteromonas phage PHS3 (GenBank accession no. KX912252, unpublished). Phosphoadenosine phosphosulfate reductases are thought to be involved in sulfate activation for cysteine biosynthesis. However, no studies have investigated the relationship between the activity of these enzymes and phage metabolism [46, 47].

Based on NCBI BLAST gene annotation results, phage vB_EliS-R6L shares 12 similar ORFs (E-value <10^-5) with the Caulobacter phage Sansa, and another 5 pairs with an E-value <10^-3 were found [42] (Table 2). The 12 homologous ORFs include 3 involved in DNA metabolism, 3 structural proteins, 1 methylase, 1 endolysin, 1 nucleoside triphosphate hydrolase, 1 molecular chaperone and 2 proteins of unknown function. However, the identities of the 12 pairs are not high (ranging from 23 to 61%; 34% on average), providing further evidence for the novelty of phage vB_EliS-R6L.

Endolysin protein (ORF 74), major capsid protein (ORF 91), portal protein (ORF 98), and terminase (ORF 100) were chosen for phylogenetic tree construction (Fig. 4). With the exception of the tree based on the terminase protein, in all cases, vB_EliS-R6L clusters with virulent bacteriophages, such as the Caulobacter phage Sansa, roseophage, and Pseudomonas phages. However, the clearly distant phylogenetic relationships with other phages suggest that vB_EliS-R6L is a novel phage. In the terminase-based tree, phage vB_EliS-R6L is located near prophages from Agrobacterium rhizogenes and Rhizobium freirei, agreeing with the BLASTp analysis. In addition, the phage life style predicted by the PHACTS algorithm indicated that it may be a temperate phage. However, no integrase, repressor, or other genes related to the SOS response [48] were identified in the genome of phage vB_EliS-R6L.

Environmental distribution

Metagenomic analysis indicated that vB_EliS-R6L-like phages are widespread in the examined environmental samples (Fig. 5). Across all metagenomic samples (JRE, POV and GOS), 7138 reads were successfully assigned and detected at rates of 10^-9 to 10^-7 per amino acid pair in the databases. The greatest matches were found in JRE (1.13 × 10^-7 per pair), from which phage vB_EliS-R6L was isolated, followed by POV (1.94 × 10^-8 per pair) and GOS (1.89 × 10^-8 per pair) coastal samples. This is in agreement with the general distribution of Erythrobacter in the coastal environment [1, 2, 49]. Forty-five ORFs were matched to homologs in the databases. The most relative abundant distribution was for ORF 49 (single-stranded DNA-binding protein, with function of DNA replication/repair, 5.28 × 10^-9 per pair), ORF 54 (hypothetical protein, 3.05 × 10^-9 per pair), ORF 32 (acyl carrier protein, 3.18 × 10^-9 per pair) and ORF 100 (terminase, 3.07 × 10^-10 per pair). Although the homologs of some ORFs (e.g., 9, 37, 45, 76, 81, 93 and 96) were only found in the JRE virome and/or the POV and GOS coastal samples, the hits for the most matched ORFs covered all three databases. This result suggests that vB_EliS-R6L is a previously unknown phage group that is widely distributed in the marine environment and that it could serve as a good reference for the taxonomic binning of marine viromes in the future.

Conclusion

Phage vB_EliS-R6L is the first virus identified that can infect marine bacteria belonging to the genus Erythrobacter. The phage has a wide temperature and pH tolerance. With a 65.7-kb genome encoding 108 putative gene products, phage vB_EliS-R6L is novel among the cultured phage community and is largely different than all other known phages. Phage vB_EliS-
R6L encodes five methylase proteins, suggesting the potential to overcome host resistance systems. Auxiliary metabolic genes in the phage genome were also annotated, such as those coding for an acyl carrier protein and phosphoadenosine phosphosulfate reductases. Metagenomic database queries suggest that vB_EliS-R6L-like phages are widely distributed in the marine environment, especially in coastal waters. Erythrobacter comprises one of the important clades of AAPBs [50, 51] and could represent the predominant AAPBs in the upper oceans [7]. Our study provides the basis for in-depth investigation of host-virus interactions and the ecological behavior of marine Erythrobacter.

**Abbreviations**
AAPB: Aerobic anoxygenic phototrophic bacteria; SM: Sodium chloride-magnesium sulfate; TEM: Transmission electron microscopy; PFU: Plaque forming unit; OD: Optical density; PCR: Polymerase chain reaction; ORFs: Open reading frames; R-M: Restriction-modification; JRE: Jiulong River Estuary; POC: Pacific Ocean Virome; GOS: Global Ocean Survey

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**Availability of data and materials**
All data generated or analysed during this study are included in this published article.

**Authors’ contributions**
LL and LC were responsible for samples collection and phage isolation. LL extracted the viral DNA, sequenced the genome, annotated the genome and carried out the phylogenetic and comparative genomic analyses. LL drafted the manuscript, and RZ, LC and NJ critically revised the manuscript. NJ and RZ organized the study. All authors have read and approved final manuscript.

**Competing interests**
The authors declare that they have no competing interests.

**Consent for publication**
Not applicable.

**Ethics approval and consent to participate**
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