

Genomics/technical resources

## Genome sequence of an inducible phage in *Rhodovulum* sp. P5 isolated from the shallow-sea hydrothermal system



Dan Lin, Kai Tang\*, Yu Han, Chenlan Li, Xiaofeng Chen

State Key Laboratory for Marine Environmental Science, Institute of Marine Microbes and Ecospheres, Xiamen University, Xiamen 361005, PR China

### ARTICLE INFO

#### Article history:

Received 22 July 2016

Received in revised form 1 October 2016

Accepted 1 October 2016

Available online 14 October 2016

#### Keywords:

Phage

Genome

Mu-like phage

Shallow-sea hydrothermal system

### ABSTRACT

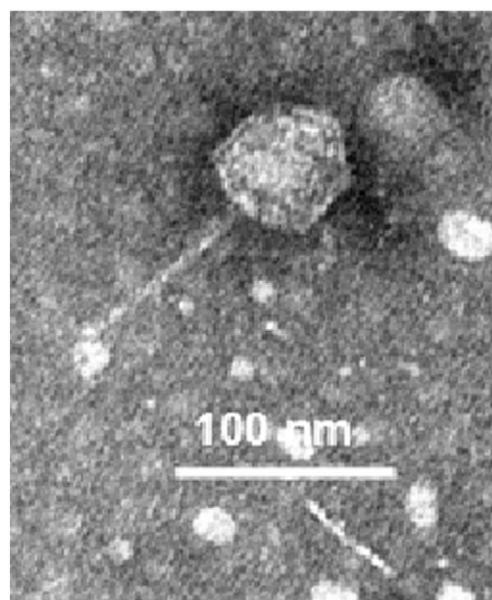
A prophage namely vB\_Rhks\_P1 was induced by mitomycin C from *Rhodovulum* sp. P5 in the shallow-sea hydrothermal systems. The vB\_Rhks\_P1 had siphovirus-like morphology, and the average particle had a head size of approximately 61 nm, and the tail length approximately 93 nm. The genome of vB\_Rhks\_P1 was a size of 38.8 kbp, 67.5% GC content, and 59 open reading frames. The genome contained Mu-like head structural genes but its genomic content was distinct from Mu or Mu-like phages.

© 2016 Elsevier B.V. All rights reserved.

### 1. Introduction

Bacteriophages are ubiquitous and abundant in the ocean, which are involved in the marine biogeochemical cycles (Weinbauer, 2004; Suttle, 2005). Phages-mediated horizontal gene transfer is a driving force in the evolution and adaptation of microbes in the marine environment (Brüssow et al., 2004; Paul, 2008). The lysogenic bacteriophages can integrate their nucleic acids into host bacterial genomes (termed prophages) and develop a symbiotic relationship with their hosts (Ackermann & DuBow, 1987). Under certain circumstances (such as UV radiation and mitomycin C treatment), the prophage can be induced from the host cell and then pass to daughter cells during cell division (Paul, 2008; Chen et al., 2006; Jiang et al., 1998; Williamson et al., 2002). Diversity of lysogenic bacteriophages has been identified in the deep-sea hydrothermal vent (Williamson et al., 2008; Millard et al., 2014). However, few investigations about phages in the shallow-sea hydrothermal fields have been conducted (at water depths of <200 m), which usually occur near active coastal or submarine volcanoes (Tang et al., 2013). The fluids of the shallow-sea vent vary considerably in temperature, pH, and chemical composition (Tarasov et al., 2005). The presence of enrichment of oxygen and nutrients compared to deep-sea vents is a profound feature of shallow hydrothermal systems (Tarasov et al., 2005). Hydrogen sulfide and elemental sulfur is naturally enriched in the shallow-sea hydrothermal fluids (Tang et al., 2013). Sulfur-oxidizer bacteria were dominant in the shallow-sea hydrothermal systems (Tang et al., 2013). The interactions between lysogenic

bacteriophages and their hosts play critical role in the ecology and evolution of bacteria in the extreme environments (Williamson et al., 2008). Presented here is the complete genome sequence of a prophage



**Fig. 1.** Electron micrographs of purified phage vB\_Rhks\_P1 particles induced from *Rhodovulum* sp. P5, with a typical siphovirus-like morphology. Scale bars: 100 nm.

\* Corresponding author.

E-mail address: [tangkai@xmu.edu.cn](mailto:tangkai@xmu.edu.cn) (K. Tang).

**Table 1**

Classification, general features and genome sequencing project information for phage vB\_RhkS\_P1 according to the MiXs recommendations (Yilmaz et al., 2011).

Item	Description
Classification	Domain: unassigned (ds DNA viruses) Order Caudovirales Family Siphoviridae
Particle shape	Isometric capsid with a flexible and non-contractile tail
Submitted to NCBI	KX077179 (GenBank)
Investigation type	Virus
Project name	Relationships between bacteria and virus
Specific host	<i>Rhodovulum</i> sp. P5 (Taxonomy ID: 1564506)
Propagation	Lysogenic
Pathogenicity	Bacteria
Geographic location	Kueishantao Islet, Taiwan, China
Environment	Shallow-sea hydrothermal systems
Latitude and longitude	24°50' N 121°57' E
Depth	25 m
Collection date	July 2015
Environment (biome)	Marine hydrothermal vent (EnvO:01000122)
Environment (feature)	Coastal water body (EnvO: 02000049)
Environment (material)	Sea water (EnvO: 00002149)
Sequencing method	Illumina HiSeq2500
Number of contigs	1
Assembly method	Velvet v1.2.03
Finishing quality	Finished (complete)
Estimated size	38.8 kbp
Assembly coverage	~266×

in *Rhodovulum* sp. P5, representative of ubiquitous sulfur-oxidizing bacteria isolated from the shallow-sea hydrothermal system in Kueishantao Islet, off Taiwan (121°57' E, 24°50' N).

## 2. Data description

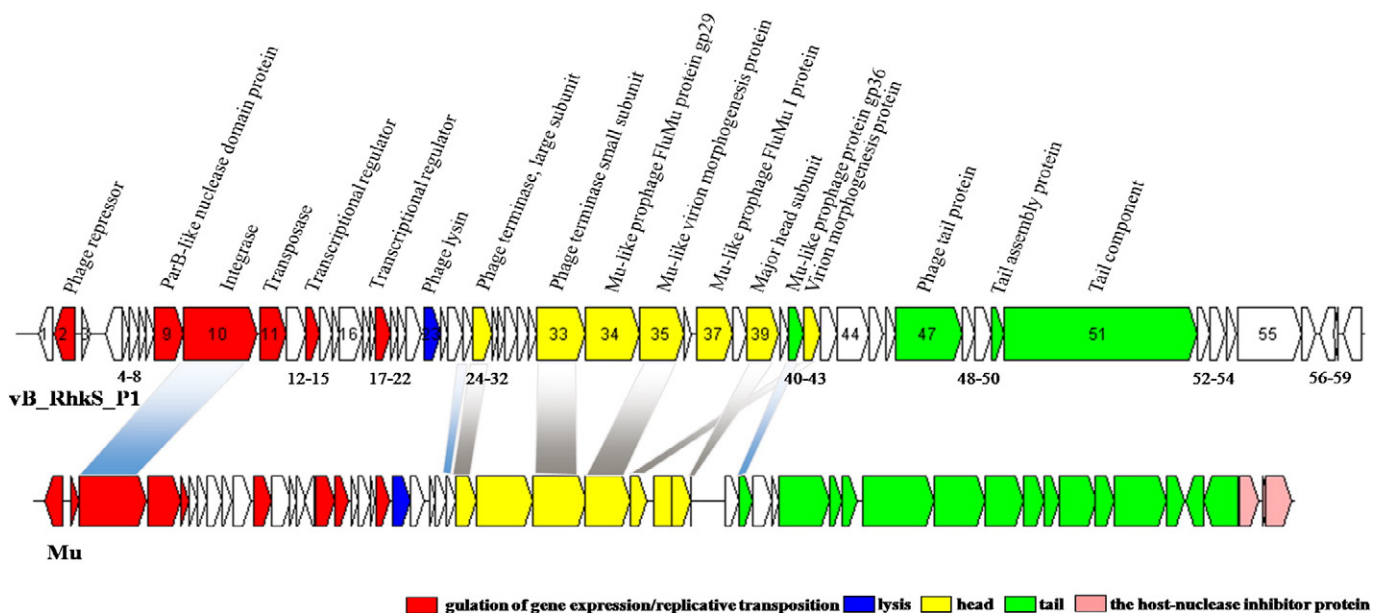
A prophage from strain P5 was induced from *Rhodovulum* sp. P5 by mitomycin C and purified by CsCl gradient centrifugation, namely vB\_RhkS\_P1 (Chen et al., 2006). Prophage showed a siphovirus-like morphology having a flexible and non-contractile tail by transmission electron microscopy (Fig. 1). The average particle had a head size of approximate 61 nm ( $n = 5$ ), and the tail length approximate 93 nm ( $n = 5$ ) (Fig. 1).

The genomic DNA of vB\_RhkS\_P1 was sequenced on Illumina HiSeq 2500 platform. The sequences were assembled using Velvet v1.2.03 (Zerbino & Birney, 2008). The genome of vB\_RhkS\_P1 contained double-stranded (ds) DNA, with a size of 38.8 kbp, and the GC content of 67.5% (Table 1). No tRNA was detected in the genome. Genome was further annotated in the RAST website server (Aziz et al., 2008). A total of 59 open reading frames (ORFs) were identified in vB\_RhkS\_P1, in which 18 ORFs were annotated as known functional genes. The genome of vB\_RhkS\_P1 could be roughly divided into four functional modules: the left half mainly encodes functions required for modulation of phage gene expression or host response and lysogeny, the right half largely encodes functions required for structure of tail and head (Fig. 2). The prophage genome harbored a repressor gene (Cro/CI family) (ORF 2), which prevents transcription and translation of lysis and other late genes (Yoshida et al., 2015). The genome possessed a ParB protein (ORF 9), a putative integrase (ORF 10) and a transposase gene (ORF 11), which function in the non-homologous recombination, and random insertion into the host genome (Denyes et al., 2014). Gp29 (ORF 34) and gp36 (ORF 41) were virulent genes (Zehr et al., 2012). The genes above mentioned acting as a “lysogeny module” are features common to temperate prophages.

The vB\_RhkS\_P1 shared 16 homologs with *Rhodobacter* phage RC1 (NC\_020839). One of the most striking annotation features of vB\_RhkS\_P1 genomes is five head structural genes are homologous with those in Mu phage. The vB\_RhkS\_P1 shared a related modular organization and similar gene content over the structural head module with Mu-like phages (Fig. 2). However, the vB\_RhkS\_P1 lacked the host-nuclease inhibitor protein, Gam, and the Mor transcription activator, which are high-frequency proteins that exit Mu and Mu-like phages (Cazares et al., 2014), whereas the ParB protein was not detected in the Mu phage. The genome sequence of vB\_RhkS\_P1 may offer a glimpse for virus in the shallow-sea hydrothermal system, providing a clue for the evolutionary of Mu and Mu-like phage.

## 3. Nucleotide sequence and strain accession numbers

The complete genome sequence of phage vB\_RhkS\_P1 has been assigned GenBank accession number KX077179. The host strain has



**Fig. 2.** Genetic organizations of vB\_RhkS\_P1 and Mu phages. The homologous genes between phages are connected by shading, the gray shade, head structural homologous genes; the blue shade, other homologous genes. Red, regulation of gene expression/replicative transposition; blue, lysis; yellow, head; green, tail; pink, the host-nuclease inhibitor protein; white, hypothetical proteins.

been deposited in Japan Collection of Microorganisms (JCM) with deposit number as JCM No. 30380.

## Acknowledgments

We thank Lanlan Cai and Yongle Xu of the Xiamen University for their useful suggestions. This work was supported by the National Natural Science Foundation of China project (41276131), State Key R&D project (2016YFA0601100), the Natural Science Foundation of Fujian Province (2014J01164), and the Fundamental Research Funds for the Central Universities (20720150078).

## References

- Ackermann, H.W., DuBow, M.S., 1987. *Viruses of Prokaryotes*. CRC press.
- Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., et al., 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9, 1.
- Brüssow, H., Canchaya, C., Hardt, W.D., 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.* 68, 560–602.
- Cazares, A., Mendoza-Hernández, G., Guarneros, G., 2014. Core and accessory genome architecture in a group of *Pseudomonas aeruginosa* Mu-like phages. *BMC Genomics* 15, 1146.
- Chen, F., Wang, K., Stewart, J., Belas, R., 2006. Induction of multiple prophages from a marine bacterium: a genomic approach. *Appl. Environ. Microbiol.* 72, 4995–5001.
- Denyes, J.M., Krell, P.J., Manderville, R.A., Ackermann, H.W., She, Y.M., Kropinski, A.M., 2014. The genome and proteome of Serratia bacteriophage η which forms unstable lysogens. *Virology* 11, 1.
- Jiang, S.C., Kellogg, C.A., Paul, J.H., 1998. Characterization of marine temperate phage-host systems isolated from Mamala Bay, Oahu, Hawaii. *Appl. Environ. Microbiol.* 64, 535–542.
- Millard, A.D., Hands-Portman, I., Zwirgmaier, K., 2014. Morphotypes of virus-like particles in two hydrothermal vent fields on the East Scotia Ridge, Antarctica. *Bacteriophage* 4, e28732.
- Paul, J.H., 2008. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? *ISME J.* 2, 579–589.
- Suttle, C.A., 2005. Viruses in the sea. *Nature* 437, 356–361.
- Tang, K., Liu, K., Jiao, N., Zhang, Y., Chen, C.T.A., 2013. Functional metagenomic investigations of microbial communities in a shallow-sea hydrothermal system. *PLoS ONE* 8, e72958.
- Tarasov, V.G., Gebruk, A.V., Mironov, A.N., Moskaliev, L.I., 2005. Deep-sea and shallow-water hydrothermal vent communities: two different phenomena? *Chem. Geol.* 224, 5–39.
- Weinbauer, M.G., 2004. Ecology of prokaryotic viruses. *FEMS Microbiol. Rev.* 28, 127–181.
- Williamson, S.J., Houchin, L.A., McDaniel, L., Paul, J.H., 2002. Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl. Environ. Microbiol.* 68, 4307–4314.
- Williamson, S.J., Cary, S.C., Williamson, K.E., Helton, R.R., Bench, S.R., Winget, D., Wommack, K.E., 2008. Lysogenic virus–host interactions predominate at deep-sea diffuse-flow hydrothermal vents. *ISME J.* 2, 1112–1121.
- Yilmaz, P., Kottmann, R., Field, D., Knight, R., Cole, J.R., Amaral-Zettler, L., et al., 2011. Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIXS) specifications. *Nat. Biotechnol.* 29, 415–420.
- Yoshida, M., Yoshida-Takashima, Y., Nunoura, T., Takai, K., 2015. Genomic characterization of a temperate phage of the psychrotolerant deep-sea bacterium *Aurantimonas* sp. *Extremophiles* 19, 49–58.
- Zehr, E.S., Tabatabai, L.B., Bayles, D.O., 2012. Genomic and proteomic characterization of SuMu, a Mu-like bacteriophage infecting *Haemophilus parasuis*. *BMC Genomics* 13, 1.
- Zerbino, D.R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18, 821–829.