Marine Genomics 44 (2019) 70–73

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

Isolation and genome sequencing of the novel marine phage PHS3 from the Yellow Sea, China

Huifang Li[a], Zhaoyang Liu[a], Min Wang[a,b,c,*], Xinxin Liu[a], Qi Wang[a], Qian Liu[a], Yong Jiang[a,b,c,*], Zhongshi Li[a], Hongbing Shao[a], Andrew McMinn[d]

[a] College of Marine Life Sciences, Ocean University of China, Qingdao, PR China
[b] Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, PR China
[c] Key Lab of Polar Oceanography and Global Ocean Change, Ocean University of China, Qingdao, PR China
[d] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

ARTICLE INFO

Keywords:
Pseudoalteromonas phage
Siphoviridae
Genome sequencing

ABSTRACT

The genus *Pseudoalteromonas* of the class Gammaproteobacteria represents a ubiquitous clade of marine bacteria, and is known to be ecologically and evolutionarily influenced by phages. Here, we report the complete genome sequence of a *Pseudoalteromonas* phage (PHS3) and major findings from the genomic analysis. Morphological observation suggested that the phage belongs to the *Siphoviridae* family. Genome of phage PHS3 consisted of a linear, double-stranded 35.626 kb DNA molecule with 40.85% GC content, and 58 putative open reading frames (ORFs) without tRNA. Comparative genome analysis revealed that phage PHS3 is related to the *Pseudoalteromonas* phage pq0 (acc. no. NC_029100). They had a synteny in the structure and DNA replication/regulation module and shared many structural and replicate genes, while little similarity was found in the phage packaging module. Knowledge of this phage might be helpful for further research on the interaction between phages and their hosts.

1. Introduction

Viruses are the most abundant and genetically diverse 'life forms' in ocean. As the major pathogens of planktonic organisms, they play an important role in nutrient and energy cycling (Suttle, 2005). Recently, investigations of viruses using metagenomics fundamentally changed our estimation of their diversity and community structure as well as our understanding of their interaction with their hosts (Brum et al., 2015; Yang et al., 2017). Although the immense genetic information provided by virome studies, most are considered "dark material" due to the lack of similarity to known sequences (Filée et al., 2005; Yang et al., 2017). It is proposed and demonstrated that this problem can be partially solved by the isolation and genetic characterization of viruses, especially those that infect dominant bacterial groups, such as *Synechococcus* and *Vibrio* in coastal areas. (Mann et al., 2005; Baudoux et al., 2012; Labrie et al., 2013; Yang et al., 2017).

Marine *Pseudoalteromonas* is an important group of bacterial genus belonging to the class Gammaproteobacteria that gained attention in the natural product and microbial ecology science fields in the last decade (Bowman, 1998). *Pseudoalteromonas* species are usually associated with eukaryotic organisms and produce a large amount of biologically active agents, such as extracellular antibiotics, toxins and polysaccharides (Qin et al., 2011). Ecologically, *Pseudoalteromonas* appears significant and to date has been shown to influence biofilm formation in various marine econiches (Dobretsov et al., 2006).

Pseudoalteromonas phages represent a significant group of phages in the ocean, which can influence the ecology and evolution of their host (Médigue et al., 2005; Thomas et al., 2008). The genomes of *Pseudoalteromonas* phages are small (3–300 kb) compared with their host (1000–13, 000 kb), and typically abound with unknown gene content (Duhaime et al., 2011). However, there are still only 23 *Pseudoalteromonas* phages genomes sequences reported in public datasets. Twenty-two of them belong to *Caudovirales*, one belongs to corticovirus (Table S1).

Here, we report the isolation and genomic characterization of PHS3, a *Siphovirus* that infects *Pseudoalteromonas*. These basic knowledges on phage are fundamental for further investigation on understanding the role of phages in marine systems and the influence of virus-host interactions on microbial mortality and genetic systems.

* Corresponding authors at: College of Marine Life Sciences, Ocean University of China, Qingdao, PR China.
E-mail addresses: mingwang@ouc.edu.cn (M. Wang), yongjiang@ouc.edu.cn (Y. Jiang).

https://doi.org/10.1016/j.margen.2018.10.001
Received 17 August 2018; Received in revised form 29 September 2018; Accepted 12 October 2018
Available online 18 October 2018
1874-7787/

Table 1
General features and genome sequencing information for phage PHS3 according to the MIxS standards.

Item	Description
Classification	Domain: unassigned (ds DNA viruses) Order *Caudovirales* Family *Siphoviridae*
Investigation type	Virus
Submitted to GenBank	KX912252.1
Particle shape	Isometric capsid with a long non-contractile tail
Geographic location	The Yellow Sea, China
Latitude and longitude	36°06′ N, 120°32′ E
Isolation source	Sea water
Depth	0.3 m
Collection date	2016-04-01
Environment biome	Temperate shelf and sea biome (ENVO: 00000895)
Environment feature	Coastal water body (ENVO: 02000049)
Environment material	Sea water (ENVO: 00002149)
Isolation and growth condition	26 °C
Sequencing method	Illumina Miseq
Number of contigs	1
Assembly method	SOAPdenovo
Finishing quality	Finish (complete)

2. Data description

The phage we studied PHS3, was isolated from the coastal water of Yellow Sea, China (36°06′N, 120°32′E) in April 2016 (Table 1). Seawater was filtered through 0.22 μm-pore-size membranes (Millipore), and phage were checked for the presence by using the double-agar layer method (0.5% low-melt top agar) to obtain the plaques (Li et al., 2016). After initial isolation, the phage was purified three times via plaque assay and harvested from plates with SM buffer (100 mM NaCl, 8.12 mM $MgSO_47H_2O$, 50 mM Tris-HCl (pH 8.0), 0.02% gelatin). Purified phage lysate was stored in SM buffer at 4 °C (Wang et al., 2016).

PHS3 formed clear plaques on the bacterial lawn of the host strain, suggesting the lytic ability of the phage. The host bacterial strain was isolated from the same place by serial dilution, and molecular identified via 16S rRNA gene sequence analysis, showing 99.93% nucleotide sequence identity to *Pseudoalteromonas marina* mano4$^{(T)}$ (acc. no. AY563031), which was also isolated from the tidal flats of the Yellow Sea (Nam et al., 2007).

For morphological characterization of PHS3, phage particles were examined at 100 kV via transmission electron microscopy (JEOL-1200 EX, Japan) after negative staining with phosphotungstic acid (2% w/v, pH 7.2) (Haq et al., 2012). The result shows that PHS3 belongs to the family *Siphoviridae,* a group of dsDNA bacteriophages in the order *Caudovirales* characterized by icosahedral head and long non-contractile tail. The head diameter and tail length of PHS3 were approximately 58 nm and 96 nm, respectively (Fig. 1).

Genomic DNA of phage PHS3 was extracted from concentrated phage particles using TIANamp virus DNA kit (Biopeony Beijing Co., Ltd., China). Phage genome was sequenced by Illumina Miseq 2 × 300 paired-end methods. Then raw data were filtered and clean reads were assembled in a single contig using SOAPdenovo (Version 2.04). The PHS3 genome was 35.626 kb in length, with 40.85% GC content. No tRNA gene was found through tRNAscan-SE searches in full genome sequence.

Gene calling and annotation using the RAST (http://rast.nmpdr.org) and Glimmer (http://ccb.jhu.edu/software/glimmer/index.shtml) server predicted 58 ORFs, with an average length of 532 bp, representing 89.7% of the entire phage sequence. Their functions were analyzed using BLAST algorithm, and the predicted proteins were compared to the NCBI non-redundant (nr) protein database to predict the functions using the BLASTP algorithm (Gong et al., 2017; Yang et al., 2017). Among the 58 protein coding genes, about 45% (26 ORFs) were functionally annotated, while 32 ORFs predicted to encode hypothetical proteins. These unknown functions ORFs could possibly be deduced from their position in the PHS3 genome.

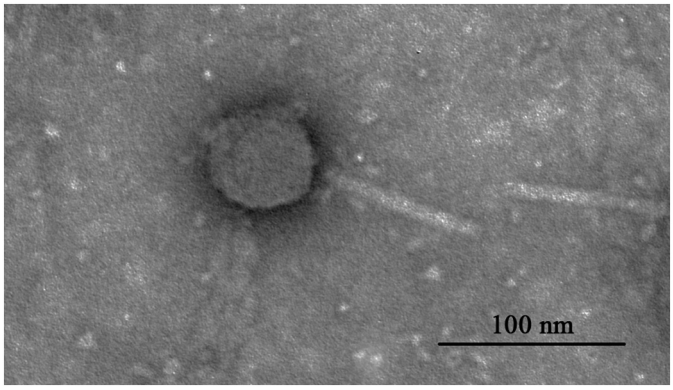

Fig. 1. Transmission electron micrograph of *Pseudoalteromonas* phage PHS3. Scale bar, 100 nm.

All the functionally annotated ORFs could be assigned a recognizable function and then grouped into four functional modules: structure, packaging, DNA replication/regulation and lysis (Fig. 2A, Table S2). Functional annotation of structural proteins largely depended on the sequence similarity to proteins of other phages that were detected in respective viral particles (Kang et al., 2016). ORF6 showed approximately 64% sequences identity with ORF5 of *Pseudoalteromonas* phages pq0, which was highly conserved and determined the tail length (Xu et al., 2004). ORF9, ORF10, ORF11, ORF13 and ORF14 showed approximately 92%,44%, 45%,43%, and 38% sequences identities with the ORF19, ORF18, ORF17, ORF16 and ORF13 of the *Idiomarinaceae* phage 1N2-2 (acc. no. NC_025439), respectively, which was isolated from the alkaline hypersaline soda Lake Nakuru in Kenya (Skvortsov et al., 2016). Terminase recognizes DNA for packaging and has a nuclease activity that is responsible for creating the ends of the virion chromosome (Catalano et al., 1995; Li et al., 2012). ORF24 coded the phage terminase large subunit, showing approximately 44% similarity to ORF2 of *Thermoanaerobacterium* phage THSA-485A (acc. no. YP_006546303.1). Most of the lytic phages code their own replication systems and replication-related enzymes (Li et al., 2012). Proteins coded by DNA replication/regulation genes in this genome included putative DNA methylase, HNH endonuclease, phosphoadenosine phosphosulfate reductase, DUF1364 domain-containing, DNA base-flipping protein, replicative DNA helicase, replication initation protein, single-strand DNA binding protein, crossover junction endodeoxyribonuclease, deoxyuridine 5′-triphosphate nucleotidohydrolase and DNA binding protein. Although most of the phages code their own conserved DNA polymerases (Chen and Schneider, 2005), there is no DNA polymerase gene identified in the PHS3 genome. Therefore, the phage PHS3 should share the DNA polymerase with the host bacteria, along with the helicase, to complete the process of phage genome replication.

Major capsid protein (MCP), one of the marker proteins of phages, was the major structural component of the icosahedral virus particles. Sequence comparison of capsid protein could provide insight into their phylogenetic relationship. The phylogenetic position of phage PHS3 was inferred using MCP (Fig. S1). MCP of PHS3 was aligned using ClustalW, together with several best BLASTP hits. Aligned MCP sequences were used to build a Neighbor-joining tree using Molecular Evolutionary Genetics Analysis software (MEGA 7.0) with 1000-fold bootstrap support. The resulting tree showed that phage PHS3 was closely related to the previously published *Pseudoalteromonas* phage pq0 (acc. no. NC_029100).

Comparison to the genome of pq0, another *Siphoviridae* lytic phage isolated from the coastal area of the Yellow Sea, China (Wang et al.,

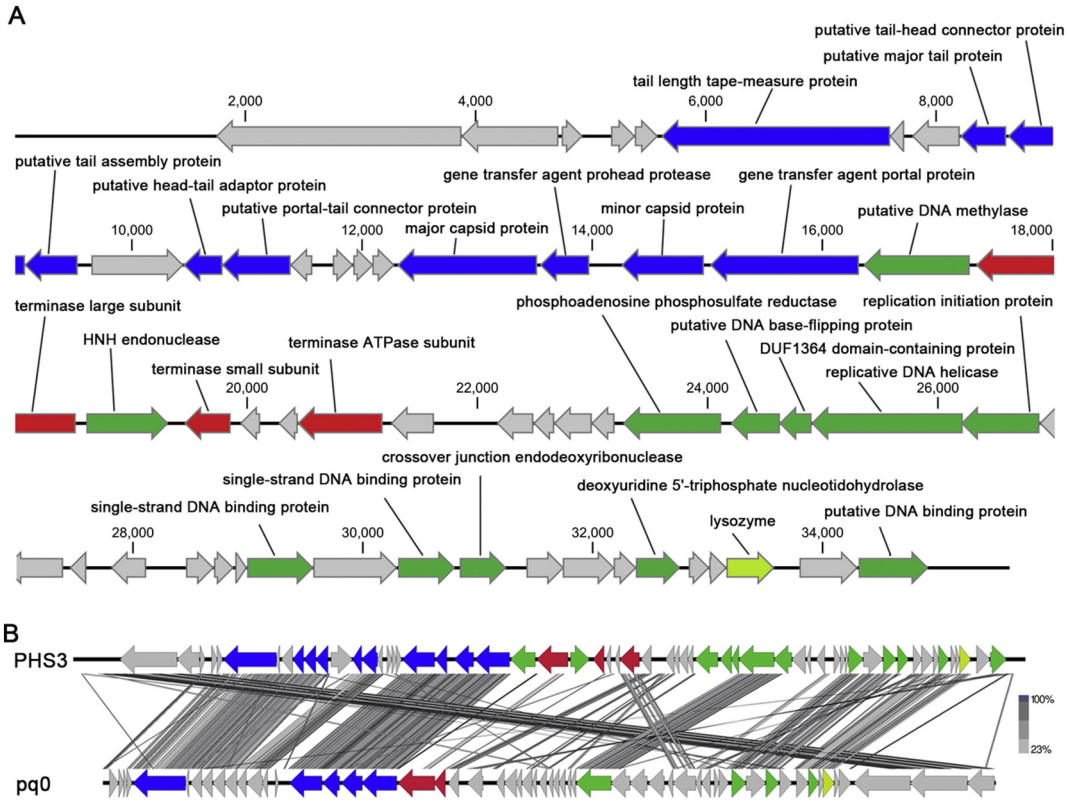

Fig. 2. Genome analysis of phage PHS3. (A) Genome map of PHS3 and functional annotation of the predicted proteins. Blue, structure; red, packaging; green, DNA replication/regulation; lime, lysis. (B) Genome-wide comparison of phages PHS3 and pq0. Genome regions showing similarity were searched using tBLASTX and the e-value ($< 10^{-3}$) cutoffs were indicated by the grey rectangle according to the color scale on the right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2016), revealed an insight into the similarities and differences between the two phages (Fig. 2B). The pq0 genome has the similar modular as PHS3 genome, with structural, DNA replication/regulation and lysis module. The two phage genomes showed synteny in those modules. ORF7, ORF8, ORF9, ORF11 and ORF12 of the pq0 genome coded the protein of unknown function was similar to the functional protein (ORF9, ORF10, ORF11, ORF13 and ORF14, respectively) of the PHS3 genome in the same direction. It may indicate that the five unknown functions ORFs were related to phage structure in pq0 genome. In contrast, they showed little similarity in the packaging modules. Moreover, homologous proteins of putative DNA methylase (ORF23), HNH endonuclease (ORF25) and putative DNA base-flipping protein (ORF36) were not predicted in pq0. This pattern of similarity, which was biased highly toward the structural module, suggested that the two phages may share similar features related to morphology while showing different aspects during replication and packaging within host cells.

In conclusion, the characterization and genome analysis of PHS3 were performed. The genetic information presented here will be helpful for further research on the interaction between marine bacteria and viruses.

3. Date availability

The complete genome sequence of *Pseudoalteromonas* phage PHS3 is available in the GenBank database under accession number KX912252.1. BioSample data are available in the NCBI BioSample database (http://www.ncbi.nlm.nih.gov/biosample/) under accession number SAMN09908246. The data have been deposited with links to BioProject accession number PRJNA487714 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2018.10.001.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC Grant Nos. 31500339, 41676178, 41076088), the National Key Basic Research Program of China (973Program, Grant No: 2013CB429704), China Postdoctoral Science Foundation (Grant Nos. 2015 M570612 and 2016T90649), and Fundamental Research Funds for the Central University of Ocean University of China (Grant Nos. 201564010 and 201512008).

References

Baudoux, A.C., Hendrix, R., Lander, G., Bailly, X., Podell, S., Paillard, C., Johnson, J., Potter, C., Carragher, B., Azam, F., 2012. Genomic and functional analysis of *Vibrio* phage SIO-2 reveals novel insights into ecology and evolution of marine siphoviruses. Environ. Microbiol. 14, 2071–2086.

Bowman, J.P., 1998. *Pseudoalteromonas* prydzensis sp. nov., a psychrotrophic, halotolerant bacterium from Antarctic Sea ice. Int. J. Syst. Evol. Microbiol. 48, 1037–1041.

Brum, J.R., Ignacio-Espinoza, J.C., Roux, S., Doulcier, G., Acinas, S.G., Alberti, A., Chaffron, S., Cruaud, C., De Vargas, C., Gasol, J.M., 2015. Patterns and ecological drivers of ocean viral communities. Science 348, 1261498.

Catalano, C.E., Cue, D., Feiss, M., 1995. Virus DNA packaging: the strategy used by phage λ. Mol. Microbiol. 16, 1075–1086.

Chen, Z., Schneider, T.D., 2005. Information theory based T7-like promoter models: classification of bacteriophages and differential evolution of promoters and their polymerases. Nucleic Acids Res. 33, 6172–6187.

Dobretsov, S., Dahms, H.-U., Qian, P.-Y., 2006. Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 22, 43–54.

Duhaime, M.B., Wichels, A., Waldmann, J., Teeling, H., Glockner, F.O., 2011. Ecogenomics and genome landscapes of marine *Pseudoalteromonas* phage H105/1.

ISME J. 5, 107–121.
Filée, J., Tétart, F., Suttle, C.A., Krisch, H., 2005. Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. Proc. Natl. Acad. Sci. U. S. A. 102, 12471–12476.
Gong, Z., Wang, M., Yang, Q., et al., 2017. Isolation and complete genome sequence of a novel *Pseudoalteromonas* phage PH357 from the Yangtze River Estuary. Curr. Microbiol. 74, 832–839.
Haq, I.U., Chaudhry, W.N., Andleeb, S., Qadri, I., 2012. Isolation and partial characterization of a virulent bacteriophage IHQ1 specific for *Aeromonas* punctata from stream water. Microb. Ecol. 63, 954–963.
Kang, I., Kang, D., Cho, J.-C., 2016. Complete genome sequence of bacteriophage P2559Y, a marine phage that infects Croceibacter atlanticus HTCC2559. Mar. Genomics 29, 35–38.
Labrie, S., Frois-Moniz, K., Osburne, M., Kelly, L., Roggensack, S., Sullivan, M., Gearin, G., Zeng, Q., Fitzgerald, M., Henn, M., 2013. Genomes of marine cyanopodoviruses reveal multiple origins of diversity. Environ. Microbiol. 15, 1356–1376.
Li, P., Chen, B., Song, Z., Song, Y., Yang, Y., Ma, P., Wang, H., Ying, J., Ren, P., Yang, L., 2012. Bioinformatic analysis of the *Acinetobacter baumannii* phage AB1 genome. Gene 507, 125–134.
Li, Y., Wang, M., Liu, Q., Song, X., Wang, D., Ma, Y., Shao, H., Jiang, Y., 2016. Complete genomic sequence of bacteriophage H188: a novel *Vibrio* kanaloae phage isolated from Yellow Sea. Curr. Microbiol. 72, 628–633.
Mann, N.H., Clokie, M.R., Millard, A., Cook, A., Wilson, W.H., Wheatley, P.J., Letarov, A., Krisch, H., 2005. The genome of S-PM2, a "photosynthetic" T4-type bacteriophage that infects marine *Synechococcus* strains. J. Bacteriol. 187, 3188–3200.
Médigue, C., Krin, E., Pascal, G., Barbe, V., Bernsel, A., Bertin, P.N., Cheung, F., Cruveiller, S., D'Amico, S., Duilio, A., 2005. Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas* haloplanktis TAC125. Genome Res. 15, 1325–1335.
Nam, Y.D., Chang, H.W., Park, J.R., Kwon, H.Y., Quan, Z.X., Park, Y.H., Lee, J.S., Yoon, J.H., Bae, J.W., 2007. Pseudoalteromonas marina sp. nov., a marine bacterium isolated from tidal flats of the Yellow Sea, and reclassification of *Pseudoalteromonas sagamiensis* as *Algicola sagamiensis* comb. nov. Int. J. Syst. Evol. Microbiol. 57, 12–18.
Qin, Q.L., Li, Y., Zhang, Y.J., Zhou, Z.M., Zhang, W.X., Chen, X.L., Zhang, X.Y., Zhou, B.C., Wang, L., Zhang, Y.Z., 2011. Comparative genomics reveals a deep-sea sediment-adapted life style of *Pseudoalteromonas* sp. SM9913. ISME J. 5, 274–284.
Skvortsov, T., de Leeuwe, C., Quinn, J.P., McGrath, J.W., Allen, C.C., McElarney, Y., Watson, C., Arkhipova, K., Lavigne, R., Kulakov, L.A., 2016. Metagenomic characterisation of the viral community of Lough Neagh, the largest freshwater lake in Ireland. PLoS ONE 11, e0150361.
Suttle, C.A., 2005. Viruses in the sea. Nature 437, 356.
Thomas, T., Evans, F.F., Schleheck, D., Mai-Prochnow, A., Burke, C., Penesyan, A., Dalisay, D.S., Stelzer-Braid, S., Saunders, N., Johnson, J., 2008. Analysis of the *Pseudoalteromonas* tunicata genome reveals properties of a surface-associated life style in the marine environment. PLoS ONE 3, e3252.
Wang, D.B., Li, Y., Sun, M.Q., Huang, J.P., Shao, H.B., Xin, Q.L., Wang, M., 2016. Complete genome of a novel *Pseudoalteromonas* phage PHq0. Curr. Microbiol. 72, 81–87.
Xu, J., Hendrix, R.W., Duda, R.L., 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. Mol. Cell 16, 11–21.
Yang, Y., Cai, L., Ma, R., Xu, Y., Tong, Y., Huang, Y., Jiao, N., Zhang, R., 2017. A novel roseosiphophage isolated from the oligotrophic South China Sea. Viruses 9.