Filamentous phage SW1 is active and influences the transcriptome of the host at high-pressure and low-temperature

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Summary

As the most abundant biological entities on the planet, viruses are involved in global biogeochemical cycles, and they have been shown to play an important role in the overall functioning of the deep-sea ecosystem. Nevertheless, little is known about whether and how deep-sea viruses affect the physiology of their bacterial hosts. Previously, the filamentous phage SW1 was identified in the bathypelagic bacterium *Shewanella piezotolerans* WP3, which was isolated from the upper sediment of West Pacific ocean. In this study, phage SW1 was shown to be active under *in situ* environmental conditions (20 MPa and 4°C) by transmission electron microscopy and reverse-transcription quantitative polymerase chain reaction. Further comparative analysis showed that SW1 had a significant influence on the growth and transcriptome of its host. The transcription of genes responsible for basic cellular activities, including the transcriptional/translational apparatus, arginine synthesis, purine metabolism and the flagellar motor, were down-regulated by the phage. Our results present the first characterization of a phage–host interaction under high-pressure and low-temperature conditions, which indicated that the phage adjusted the energy utilization strategy of the host for improved survival in deep-sea environments.

Introduction

Believed to be the most abundant biological agents in the ocean (Suttle, 2007), viruses play an important role in global nutrient and biogeochemical cycles, deep-sea microbial metabolism and overall functioning of the marine ecosystem (Danovaro *et al*., 2008; Dell'Anno *et al*., 2015). Lysogeny has been suggested to be the main viral proliferation mode in the deep-subseafloor (Engelhardt *et al*., 2012) and the majority of microorganisms living in this environment are characterized by an extremely low rate of metabolism and growth, with doubling times of 100–1000 years, which has challenged our current understanding of microbial life (Hoehler and Jørgensen, 2013). However, no viruses isolated from the low-temperature and high-pressure deep-sea biosphere have been studied. Thus, both the status and the activity of these viruses and their relationship with the prokaryotic host are largely unknown.

Previously, we isolated the filamentous phage (Inovirus) SW1 from a deep-sea bacterium,

For viruses and its infecting microorganisms, the environmental adaptation should be a significant driving force of shaping different life style and regulatory mechanism. From this respect, the activity of deep-sea sediment phage SW1 and its influence on bacterial host are worth to be investigated although some marine or terrestrial isolated phages have been studied. Our previous study has shown that the bathypelagic filamentous phage SW1 have a significant influence on transcription of 49 genes (including 16 lateral flagellar genes) and swarming motility of WP3 at 4°C (Jian *et al*., 2013). In this study, the activity of SW1 was shown to significantly affect the growth and transcriptome of its deep-sea bacterial host. To our knowledge, this is the first investigation of deep-sea phage and host interactions under *in situ* environmental conditions. Our findings will contribute to the better understanding of the status of benthic bacteriophages and its influence on microorganisms in the deep biosphere.

Results and discussion

SW1 genes are homologous to genes from other filamentous phages from cold environments

At present, 42 genomes belonging to *Inoviridae* have been documented in the NCBI genome database (http://www.ncbi.nlm.nih.gov/genome). However, most of these Inoviruses are host-associated, including *Vibrio* (13), *Ralstonia* (10), *Enterobacteria* (6), *Spiroplasma* (4), *Stenotrophomonas* (2), *Pseudomonas* (2), *Acholeplasma* (1) and uncultured (3). To testify whether the Inoviruses gene homology are related with their isolation environments, comparative genomic analysis was performed to compare SW1 and other filamentous phages that were isolated from various environments (Fig. 1). Significantly higher similarities were observed between SW1 and f327 (infecting *Pseudoalteromonas* sp. BSi20327) compared with CTXΦ or φSHP1, which infect *Vibrio cholera* and *Stenotrophomonas maltophilia*, respectively (McLeod *et al*., 2005; Liu *et al*., 2012; Yu *et al*., 2015). Moreover, the major capsid protein of SW1 shares the highest homology (60%) with a protein in the prophage of *Colwellia psychrerythraea* 34H, which was isolated from Arctic sediment (Wells, 2008). These data indicate that filamentous phages from cold marine environments share a high degree of homology and may share similar regulatory mechanisms, which could be inferred by the high identity (78%) between regulators in SW1 and f327. Recent evidence indicates the prevalence and potential ecological function of filamentous phages in diverse environments. However, there is no report of Inovirus isolated from high-pressure habitats, so it remains unclear whether and how they affect the physiology of their bacterial host within the deep biosphere.

SW1 is active under low-temperature and high-pressure conditions

Similar with other filamentous phages, the release of SW1 does not lead to lysis of its bacterial host. However, turbid plaque can be formed on *Shewanella psychrophila* WP2 (the sensitive bacterial host) cell lawns after SW1 infection, which was speculated to influence the growth of *S. psychrophila* WP2 (Wang *et al*., 2007). In earlier studies, we observed that the replication of filamentous phage SW1 is active at low temperature (1.1×10^4 and 9.6×10^4 plaque-forming units at 15 and 4°C, respectively) (Wang *et al*., 2007; Jian *et al*., 2012), but whether life cycle of SW1 is active in benthic environment has yet to be determined. Initially, WP3 was cultured under both optimal growth conditions (0.1 MPa and 20°C) and *in situ* environmental conditions (20 MPa and 4°C), and the expression of SW1 genes was assessed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) (see Supplementary Methods in Supporting Information). The transcriptional levels of *fpsABE*, which encodes a replication protein, an ssDNA-binding protein and a major capsid protein, respectively, increased significantly (25- to 80-fold) at high pressure and low temperatures (Fig. 2A). Furthermore, the TEM results showed that filamentous phage particles were secreted from host cells that were cultivated at 20 MPa and 4°C, indicating that SW1 is active under this condition (Fig. 2B).

SW1 influences the growth and gene transcription of host cells

Although SW1 was not observed to influence the growth of WP3 either at 20 or 4°C under ambient pressure (Jian *et al*., 2013), a significantly higher growth rate ($0.068\ h^{-1}$) of the phage-free strain (WP3ΔSW1) was observed compared with the WP3 wild-type strain ($0.047\ h^{-1}$) at 20 MPa and 4°C (Supporting Information Fig. S1), which suggests that the production of virus particles exerts a burden on WP3 growth in the deep-sea environment. It could provide one answer to an important question: is the virus-host relationship a burden or a benefit to the host population (Sandaa, 2008)? There is an energy cost to carrying additional biological entities for the host, but the bacterium can also benefit from the phage-mediated lateral gene transfer (Breitbart, 2012). The effect of the phage on the bacterial transcriptome was investigated in a whole-genome microarray analysis (see Supplementary Methods in Supporting Information). The correlation coefficient (R^2) between the data obtained by microarray and qPCR was 0.9626 (Supporting Information Table S1 and Fig. S2), thus demonstrating that the microarray data were reliable and could be used for the follow-up analysis. The microarray

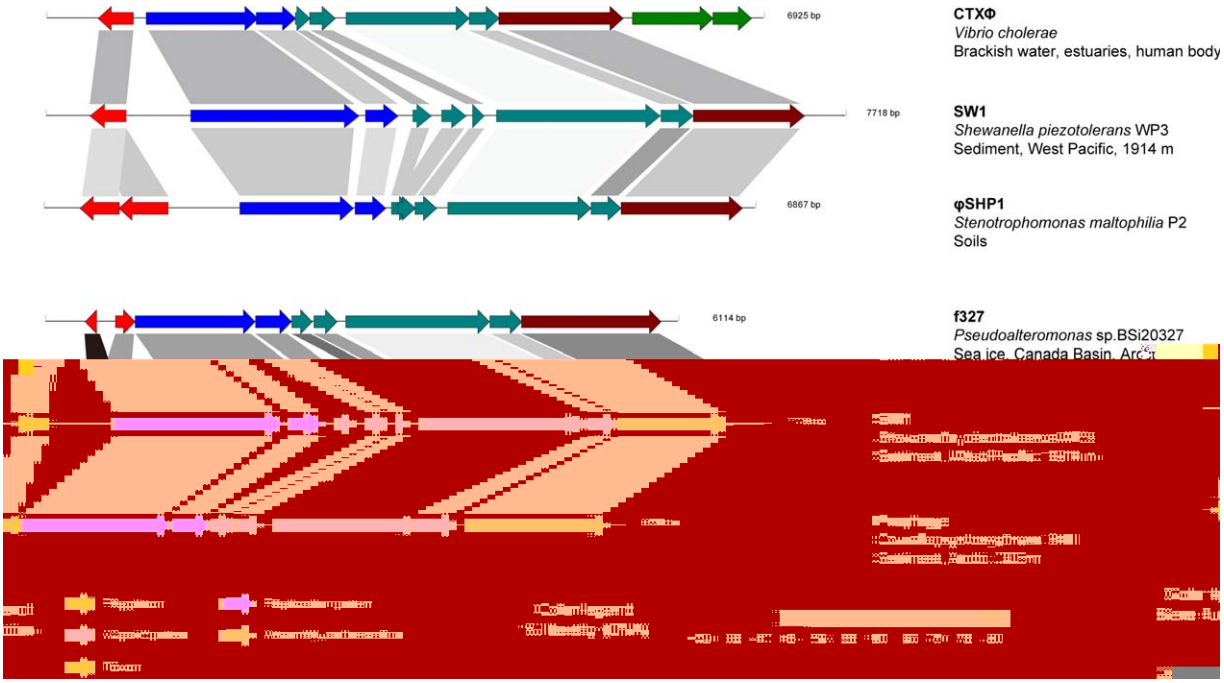

Fig. 1. Comparative genomic analysis of SW1 and other filamentous phages. The scale at the top of the genome is in base pairs. Each arrow represents an ORF, with the colour representing the function of the encoded protein that is indicated in the legend. Percent identities (nucleic acids) between adjacent genomes are coloured as denoted at the bottom of the figure, with some percentages noted. The host bacterium and natural environment are indicated on the right.

data have been deposited in NCBI's Gene Expression Omnibus (series accession number GSE66462).

Overall, 89 genes were found to be differentially expressed between the WP3 and WP3ΔSW1 at 20 MPa and 4°C, and they accounted for 1.74% of the total open reading frames (ORFs) of WP3 (Supporting Information Fig. S3, Tables S2 and S3). Twelve genes that were associated with the basic transcriptional and translational apparatus were upregulated in WP3ΔSW1 (Fig. 3), including two components for RNA polymerase (*rpoB* and *rpoC*), ribosomal proteins (*rplORQ, rpsKHEM*), rRNA methylase (*rlmB*), ribosome-binding factor A (*rbfA*) and pseudouridine synthase (*rsuA*), which suggests that WP3 reduces the production of mRNA and proteins once it is infected. Moreover, several genes involved in arginine biosynthesis (*argBCFG*), purine metabolism (*guaAB*) and Na (+)-translocation (*nqrBE*) also showed increased transcription in the phage-free strain. Because L-arginine not only is one

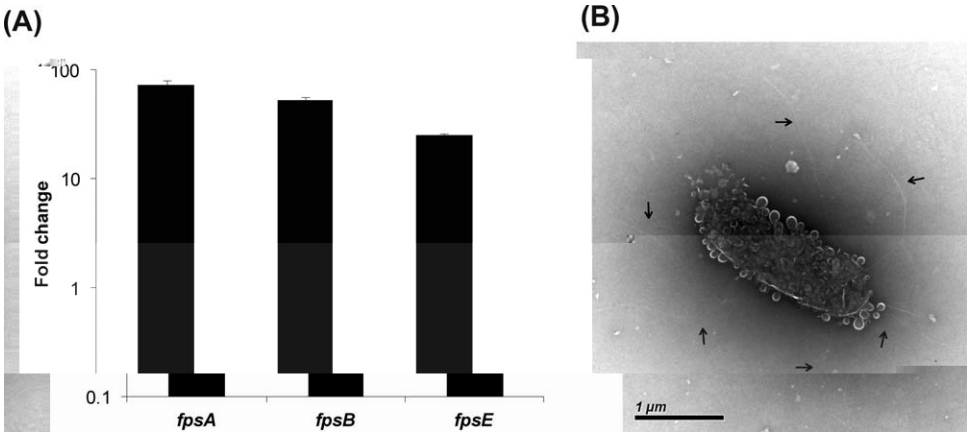

Fig. 2. SW1 is active at low temperature and high pressures. A. Changes in expression of the SW1 genes *fpsA* (replication protein), *fpsB* (ssDNA-binding protein) and *fpsE* (major capsid protein) at 20 MPa and 4°C. Gene expression of these genes at 0.1 MPa and 20°C was set as 1. The data shown represent two independent experiments, and the error bars indicate standard deviations. B. Transmission electron microscopic observation of WP3 cells showing filamentous phage particles that were secreted from the cell surface. The WP3 cells were cultivated at 20 MPa and 4°C, and the arrows indicate the presence of a filamentous phage.

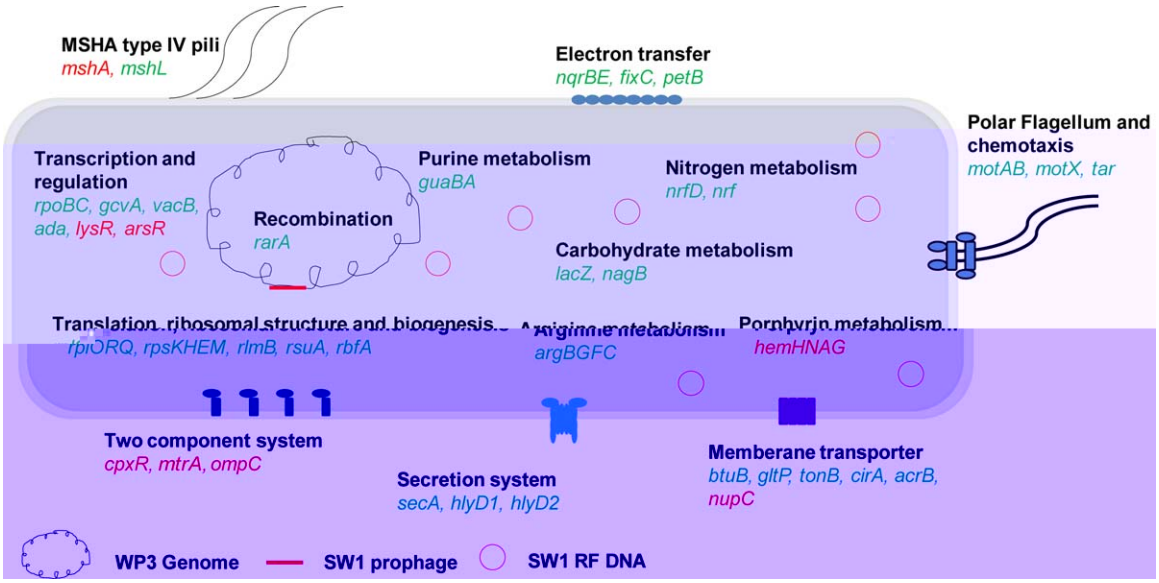

Fig. 3. Graphic display of microarray data for genes categorized by function in the phage–host interaction at 20 MPa and 4°C. Green and red indicate genes which are induced and repressed (WP3ΔSW1/WP3), respectively. The presence of the prophage and double-stranded replicative form of SW1 are demonstrated as red line and circles, respectively.

of the building block of proteins and a precursor of polyamine synthesis but also serves as a source of carbon, nitrogen and energy through a variety of catabolic pathways in bacteria (Lu, 2006), SW1 was suggested to influence the metabolism of WP3 by suppressing the production of arginine. In addition, inosine monophosphate (IMP) dehydrogenase and guanosine monophosphate (GMP) synthase (encoded by *guaB* and *guaA*) catalyze the limiting step in guanine nucleotide biosynthesis, which is essential for DNA and RNA synthesis. Thus, the downregulation of purine metabolism is likely responsible for the growth deficiency of WP3 at 20 MPa and 4°C.

Interestingly, two polar flagellar genes, *motA1* and *motB2*, had increased expression. Nevertheless, the transcription of lateral flagellar genes was not significantly changed (Supporting Information Fig. S4). This result is different from the case at 0.1 MPa/4°C, which showed that the transcription of lateral flagellar genes was decreased in WP3ΔSW1 (Jian *et al.*, 2013). Because bacterial motility using flagella is very expensive for the cell in terms of the large amount of energy consumed for flagellar biosynthesis and function (Soutourina and Bertin, 2003), downregulating the key genes of the polar flagellum while maintaining swarming motility may be an adaptive strategy for WP3 living in energy-limited deep-sea sediment.

The involvement of SW1 in adjusting the life strategy of deep-sea bacteria

It has been suggested that ~ 46% of bacterial isolates from the sub-surface harbour prophages (Danovaro *et al.*, 2008; Engelhardt *et al.*, 2011). Temperate phages, which do not kill the microbial cells, may be more abundant (such as in deep-sea hydrothermal plumes (Williamson *et al.*, 2008)) and play more important roles in community evolution (Anderson *et al.*, 2011). In our present work, we conducted the first investigation of phage-bacterium interactions under *in situ* environmental conditions, which demonstrated a distinct gene expression profile compared with cold cultivation at ambient pressure (Supporting Information Fig. S5). More differentially expressed genes were found in WP3ΔSW1 at 20 MPa and 4°C (Supporting Information Fig. S3). These results indicated that phage–host interactions was significant influenced by environmental factor such as hydrostatic pressure. Additionally, two genes (*swp0891* and *swp4118*) and two genes (*swp0264* and *swp0265*) were shown to be up-regulated and down-regulated in WP3ΔSW1 under both environmental conditions, respectively (Supporting Information Fig. S5). It would be interesting to characterize the function of these genes in the phage–host interactions in future study.

The deep-sea phage SW1 was shown to be active and to confer a fitness cost for bacterial growth while adjusting the gene expression associated with energy production and metabolic processes, including anabolism and motility. Thus, the saved energy would compensate for phage production and host survival under extreme conditions. Meanwhile, the reduced growth rate indicated a putative role of the virus in adjusting the ecological strategy of the microorganisms from a relatively rapidly growing to a slow growing life style, which would be more advantageous for resistance to adverse environments (Suttle, 2007). The phage SW1 could maintain the WP3 population at appropriate levels in the deep-

ocean sediment, which is characterized by limited nutrients and energy resources, and is thus beneficial for the survival of the community.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. The Growth curve of WP3 and WP3ΔSW1 at 20 MPa and 4°C.

Fig. S2. A correlation analysis of microarray and RT-qPCR assays.

Fig. S3. Number and functional annotation of the differentially expressed genes according to their COG categories.

Fig. S4. An assay of relative transcriptional levels of polar and lateral flagellar genes in the SW1 mutant at 20 MPa and 4°C.

Fig. S5. Hierarchical cluster plot showing the differentially expressed genes in WP3ΔSW1 under different culture conditions.

Table S1. Real-time qPCR primers used in this study.

Table S2. Up-regulated genes in WP3ΔSW1 at 20 MPa and 4°C.

Table S3. Down-regulated genes in WP3ΔSW1 at 20 MPa and 4°C.