Curr Microbiol (2016) 72:120–127
DOI 10.1007/s00284-015-0926-3

Isolation and Characterization of the Lytic Cold-Active Bacteriophage MYSP06 from the Mingyong Glacier in China

Mingyuan Li[1,2] · Jilian Wang[1] · Qi Zhang[2] · Lianbing Lin[2] · Anxin Kuang[3] · Luis Alberto Materon[3] · Xiuling Ji[2] · Yunlin Wei[2]

Received: 14 June 2015 / Accepted: 8 September 2015 / Published online: 23 October 2015
© Springer Science+Business Media New York 2015

Abstract As unique ecological systems, glaciers are characterized by low temperatures and low nutrient levels, which allow them to be considered as "living fossils" for the purpose of researching the evolution of life and the environmental evolution of the earth. Glaciers are also natural microbial "reservoirs". In this work, a lytic cold-active bacteriophage designated MYSP06 was isolated from *Janthinobacterium* sp. MYB06 from the Mingyong Glacier in China, and its major characteristics were determined. Electron microscopy revealed that bacteriophage MYSP06 had an isometric head (74 nm) and a long tail (10 nm in width, 210 nm in length). It was classified as a *Siphoviridae* with an approximate genome size of 65–70 kb. A one-step growth curve revealed that the latent and burst periods were 95 and 65 min, respectively, with an average burst size of 16 bacteriophage particles per infected cell. The bacteriophage particles (100 %) adsorbed to the host cells within 10 min after infection. Moreover, the pH value and thermal stability of bacteriophage MYSP06 were also investigated. The maximum stability of the bacteriophage was observed at the optimal pH 7.0, and the bacteriophage became completely unstable at the extremely alkaline pH 11.0; however, it was comparatively stable at the acidic alkaline pH 6.0. As MYSP06 is a cold-active bacteriophage with a lower production temperature, its characterization and its relationship with its host *Janthinobacterium* sp. MYB06 deserve further study.

✉ Xiuling Ji
jixiuling1023@126.com

✉ Yunlin Wei
weiyunlin18@gmail.com

[1] Key Laboratory of Ecology and Biological Resources in Yarkand Oasis, Kashgar University, Kashi 844006, Xinjiang, China

[2] Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650091, Yunnan, China

[3] Department of Biology, The University of Texas-Pan American, Edinburg, TX 78539, USA

Introduction

Glacier microorganisms contain abundant information pertaining to the evolution of life, and the study of glacier microorganisms will provide new ideas for the study of genetic diversity, evolution, and response mechanisms to environmental change. Investigations of cold-active bacteriophages have increased recently [12, 18]. Cold-active bacteriophages are viruses that are parasitic to bacteria and are capable of infection and production at temperatures of ≤4 °C [29]. As the most abundant life forms on earth, bacteriophages not only participate in the microbial food chain and play a significant role in regulating population structure, the diversity of microbial communities, and genetic transformation but also influence the interactions between organisms and the environment, particularly in the global biogeochemical cycle [1, 11, 20, 27, 28, 30]. As an ideal model system, the cold-active bacteriophages can be used to study the basic biological properties and cold adaptation mechanisms of cold-adapted microorganisms [7–9]. In the past few years, bacteriophages isolated from polar regions, the deep sea, permafrost regions, and high latitude lakes have been described [23]. However, thus far, culture-dependent low-latitude glacier phages have not been reported.

Glaciers constitute a unique ecological system. The Mingyong Glacier is located in the Meili Snow Mountains,

which belong to the Hengduan Mountains and demonstrate the highest biodiversity in Yunnan Province, China [31]. Studying the Mingyong Glacier psychrophilic bacteria and their phages not only enriches biodiversity research but will also provide significant guidance in revealing regional microbial community succession laws and unveiling psychrophilic mechanisms. In this study, a new cold-active bacteriophage, MYSP06, which is specific for the *Janthinobacterium* sp. MYB06 strain, was isolated from the Mingyong Glacier and its major characteristics were determined.

Materials and Methods

Sampling and Bacteriophage Isolation

Culture Medium

The PYGV medium used in this study was prepared as follows: 10 g/L tryptone, 5 g/L yeast extract, 20 mL/L Hutner's basal salts solution, 10 mL/L 25 % D-glucose, and 5 mL/L 2× vitamin solution. Hutner's basal salts solution contained 29.7 g/L $MgSO_4{\cdot}7H_2O$, 10 g/L nitrilotriacetic acid, 3.35 g/L $CaCl_2{\cdot}2H_2O$, 99 mg/L $FeSO_4{\cdot}7H_2O$, 9.25 mg/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 50 mL/L Metals 44 solution. Metals 44 solution contained 10.95 g/L $ZnSO_4{\cdot}7H_2O$, 5 g/L $FeSO_4{\cdot}7H_2O$, 2.5 g/L sodium EDTA, 1.54 g/L $MnSO_4{\cdot}H_2O$, 392 mg/L $CuSO_4{\cdot}5H_2O$, 248 mg/L $Co(NO_3)_2{\cdot}6H_2O$, and 177 mg/L $Na_2B_4O_7{\cdot}10H_2O$. The 2× vitamin solution contained 20 mg/L pyridoxine·HCl, 10 mg/L p-aminobenzoic acid, 10 mg/L calcium DL-pantothenate, 10 mg/L nicotinamide, 10 mg/L riboflavin, 10 mg/L thiamine·HCl, 4 mg/L biotin, 4 mg/L folic acid, and 0.2 mg/L vitamin B12. The pH value was adjusted to 6.0. To prepare the solid or semi-solid medium, 15 g of agar or 4 g of agarose was added to the medium, respectively.

Bacterial Strains

Water samples were obtained from the Mingyong Glacier (98°81′18.4″E, 28°47′33.0″N, altitude 2066 m, 4 °C and pH 6.0), located in Yunnan Province, China. The samples were spread on PYGV solid plates and incubated for 2–3 days at 15 °C. Purple-pigment-producing colonies were selected and were purified by streaking them on fresh plates. The molecular identification of the isolates was obtained using 16S rRNA gene sequence analysis. The PCR primers used were as follows: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [14]. The 1.5-kb PCR product was sub-cloned into the pMD-18T vector (Takara, Dalian) and was sequenced by the Beijing Sangon Biological Engineering Technology and Service in China.

Isolation of the Bacteriophage

Strain MYB06 was used as the host to isolate, propagate, and characterize the bacteriophage. Fifty milliliters of a water sample collected from the Mingyong Glacier was pre-incubated with the host cells (MYB06) to enrich the bacteriophages. After incubation at 15 °C for 15 days, the culture was centrifuged (13,000×*g*, 5 min at 4 °C, Beckman Avanti J-25, Fullerton, CA, USA), and the supernatant was filtered through a 0.22-μm filter (Millipore Corp., Bedford, MA, USA).

Bacteriophages were obtained via successive single-plaque isolation using the double-layer agar method [21]. One-hundred microliters of the bacteriophage stock was mixed with 300 μL of the MYB06 host cell culture (OD_{600} = 0.4–0.5). After incubation at 15 °C for 10 min, the mixture was added to 3 mL of liquid semi-solid medium (0.4 % agarose in PYGV liquid medium), mixed gently, and poured onto a PYGV solid plate. The double-layer plates were incubated right-side up at 15 °C overnight to count the plaques.

Purification of Bacteriophage Particles

The bacteriophage particles were purified according to the method of Stenholm et al. [25] and LaFrentz et al. [13]. The bacteriophages were purified by successive single-plaque isolation. A culture containing 2 × 10^9 PFU/mL bacteriophage particles was centrifuged (12,000×*g*, 30 min at 4 °C, Beckman Avanti J-25) to remove the cells. DNase I and RNase A (Sigma-Aldrich, St. Louis, MO, USA) were added to the supernatant to a final concentration of 1 μg/mL. The mixture was incubated at 37 °C for 30 min. To precipitate the bacteriophage particles, PEG 8000 and NaCl were added to final concentrations of 10 % (w/v) and 1 M in the supernatant, respectively. After incubation on ice for 24 h, the bacteriophage particles were pelleted by centrifugation (11,000×*g*, 15 min at 4 °C, Beckman Avanti J-25, CA, USA), and then resuspended in SM buffer (100 mM NaCl, 50 mM Tris–HCl pH 7.5, 10 mM $MgSO_4$, and 0.01 % gelatin), followed by extraction with chloroform and centrifugation (11,000×*g*, 15 min at 4 °C, Beckman Avanti J-25). To further purify the bacteriophage particles, solid CsCl was added to the supernatant to a concentration of 0.45 g/mL for ultracentrifugation (40,000 rpm, 24 h at 4 °C, SW41 rotor, Beckman LE-80K). The bacteriophage band was collected and was dialyzed against SM buffer (100 mM NaCl, 50 mM Tris–HCl pH 7.5, and 10 mM $MgSO_4$) at 4 °C. The bacteriophage particles were detected using the double-layer agar technique [28].

Electron Microscopy

The morphology of the bacteriophages was examined by performing transmission electron microscopy (TEM) of negatively stained preparations. A drop of approximately 10^{11} PFU/mL was applied to the surface of a formvar-coated grid (200-mesh copper grids), which was negatively stained using 2 % (wt/vol) uranyl acetate, and then examined in a JEM-1400 transmission electron microscope operated at 80 kV (JEOL, Tokyo, Japan).

Adsorption Experiments

The bacteriophage adsorption experiments were performed as described by Lin et al. [17] and Shen et al. [24]. *Janthinobacterium* sp. MYB06 cells (3×10^8 cfu/mL) were infected with bacteriophage at a multiplicity of infection (MOI) of 10 and were incubated at 15 °C. Aliquots of 100 µL were taken at 0, 2, 4, 6, 8, 10, 15, 20, and 30 min after infection, diluted in 0.9 mL of cold PYGV, and centrifuged (12,000×g, 5 min), after which the supernatants containing the unadsorbed bacteriophages were titrated using the double-layer agar method.

One-Step Growth Curve

A bacteriophage sample was mixed with 1 mL of fresh MYB06 host cells (3×10^8 cfu/mL) to obtain an MOI of 10 and allowed to adsorb for 15 min at 15 °C [19, 29]. The mixture was centrifuged (13,000×*g*, 2 min), and the pellets were suspended in 5 mL of fresh medium. The cell suspension was shaken at 15 °C. The bacteriophage titers in the culture were measured using the double-layer agar method at 10-min intervals. The plates were incubated at 15 °C overnight to evaluate the development of plaques. The assays were performed in triplicate.

Effect of Temperature on Phage Production

To investigate the effect of temperature on phage production, 100 µL of strain MYB06 (1×10^8 cfu/mL) was infected with MYSP06 at an MOI of 0.1. After incubation at 15 °C for 10 min, shaking culture were grown for 6 h at different temperatures (4, 8, 10, 12, 15, 18, 20, or 25 °C) in 6 mL of liquid medium. The phage titer in the culture was determined using the double-layer agar method.

Thermolability and pH Sensitivity of the Cold-Active Bacteriophage

To examine the thermolability of the cold-active bacteriophage, the bacteriophage stock (1×10^8 PFU/mL) was incubated at 40, 50, 60, or 70 °C for 1 h. The rate of survival of each treated bacteriophage sample was determined at 10-min intervals using the double-layer agar technique [6]. The plates were incubated at 15 °C overnight to evaluate the development of plaques. The assays were performed in triplicate.

To maintain a constant pH, 10 µL of the bacteriophage stock (1×10^8 PFU/mL) was added to 0.99 mL of modified liquid medium (the pH values were adjusted to between 3 and 11 by adding 1 M HCl or 1 M NaOH) and was incubated for 1 h at room temperature. The rates of survival of the bacteriophage samples were determined using the double-layer agar technique. The plates were incubated at 15 °C overnight to evaluate the development of plaques.

Sensitivity to Organic Solvents, Detergents, and Proteolytic Degradation

To study bacteriophage sensitivity to organic solvents and detergents, the bacteriophage particles were exposed to 25 % (wt/vol) chloroform for 10 min, to 0.3 % (wt/vol) Triton X-100 for 10 min at room temperature and to 0.1 % (wt/vol) sodium dodecyl sulfate (SDS) for 6 min at 50 °C. The bacteriophage sensitivity to proteolytic degradation was determined by incubating the bacteriophage particles with proteinase K (1 mg/mL, Shanghai Sangon, China) in buffer (10 mM Tris–HCl, 5 mM EDTA) for 1 h at 56 °C [10]. The number of surviving bacteriophages was determined using the double-layer agar technique. The plates were incubated at 15 °C overnight to evaluate the development of plaques.

Extraction and Restriction Endonuclease Digestion of Bacteriophage DNA

EDTA, SDS, and proteinase K were added to the CsCl-purified bacteriophage suspension to final concentrations of 20 mM, 10 %, and 50 g/mL, respectively. After incubation at 56 °C for 3 h, the mixture was extracted using phenol/chloroform, and the DNA was precipitated using ethanol [26]. The air-dried DNA pellet was dissolved in TE buffer (10 mM Tris–HCl, and 5 mM EDTA, pH 8.0). The DNA was digested using *Eco*R1, *Bam*H1, *Pst*1, and *Xho*1 (TaKaRa, Dalian, China) [22].

Protein Analysis

The CsCl-purified bacteriophage particles were mixed with loading buffer, heated in boiling water for 5 min, and then subjected to SDS-PAGE (12 %) [32]. The protein bands were stained using Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Hercules, CA, USA).

Results

Isolation and Morphology

Based on the morphology, physiology and 16S rRNA gene sequence phylogenetic analysis, strain MYB06 was identified as a member of the *Janthinobacterium* sp. (GenBank accession number KJ561889). *Janthinobacterium* sp. MYB06 was found to be an aerobic, Gram-negative, rod-shaped, purple-pigment producing, non-spore forming, and psychrophilic bacterium that grows at 4–30 °C, with optimal growth occurring at 10–20 °C.

A bacteriophage infecting *Janthinobacterium* sp. MYB06 was isolated and was named MYSP06 (Mingyong *Siphoviridae* Bacteriophage 06). Clear plaques of 1–1.5 mm in diameter were obtained after incubating the bacteriophage at 4 °C for 48 h when assayed on a double-layer agar plate; therefore, MYSP06 was a typical cold-active bacteriophage. TEM analysis showed that MYSP06 had an icosahedral head of 74 nm in diameter and a tail that was 210 nm in length and 10 nm in width (Fig. 1), characteristic of members of the family *Siphoviridae*.

Bacteriophage Production

The Temperature Range of Plaque Formation

Following infection with bacteriophage MYSP06, *Janthinobacterium* sp. MYB06 was able to produce progeny bacteriophage particles when incubated at temperatures between 4 and 20 °C. The bacteriophage production occurred optimally at 15 °C.

Adsorption Experiments

Bacteriophage MYSP06 adsorbs efficiently, has a short latent period and causes complete lysis. The adsorption rates of bacteriophage MYSP06 to *Janthinobacterium* sp. MYB06 cells are shown in Fig. 2; approximately 80 % of the bacteriophage particles adsorbed to the host cells within 2 min, 95 % within 4 min, and nearly 100 % within 10 min.

Following infection with bacteriophage MYSP06, *Janthinobacterium* MYB06 was able to produce progeny phage particles when cultured at 4–20 °C. The phage titer reached a maximum at 15 °C (Fig. 3). The one-step growth curve revealed that the latent and burst periods were 95 and 65 min, respectively, with an average burst size of 16 bacteriophage particles per infected cell (Fig. 4).

Thermolability and pH Sensitivity

Thermolability was the salient physical feature of the cold-active bacteriophage MYSP06 (Fig. 5). However, the ability of MYSP06 to infect the host decreased rapidly when the temperature exceeded 50 °C. MYSP06 was most stable at pH 3.0–9.0 but was sensitive when the pH value exceeded 9. The pH range for survival ranged from 3 to 10 (Fig. 6).

Sensitivity to Chloroform, Detergents, and Proteolytic Degradation

Bacteriophage MYSP06 was shown to be insensitive to chloroform and Triton X-100 but was completely destroyed by treatment with proteinase K (1 mg/mL) for 1 h, as well as by incubation with 0.1 % SDS. These results suggested that the capsid of MYSP06 did not contain lipids.

Analysis of the Bacteriophage DNA and Proteins

MYSP06 DNA was isolated and digested using *Eco*R1, *Bam*H1, *Pst*1, and *Xho*1 (Fig. 7). The patterns of digestion showed that MYSP06 contains double-stranded DNA with an estimated size of approximately 65–70 kb. The purified bacteriophage particles were subjected to SDS-PAGE

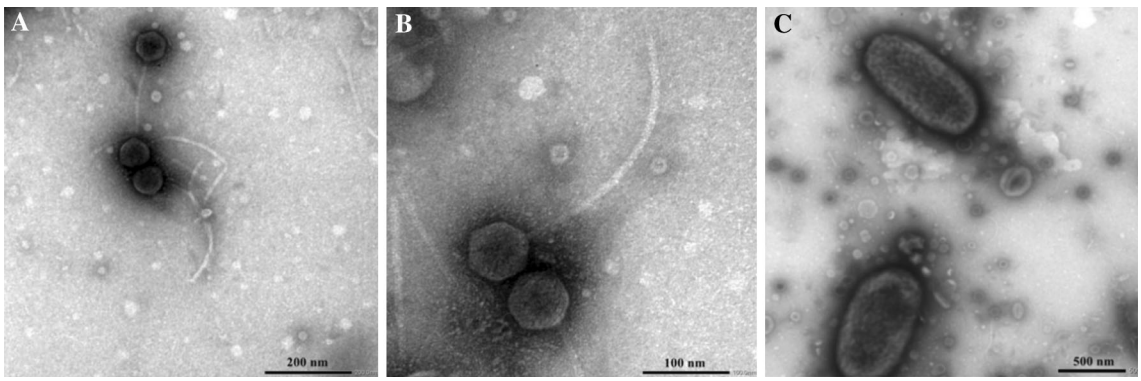

Fig. 1 Transmission electron micrographs of MYSP06 and its host cells. **a**, **b** Isolated phage MYSP06. **c** MYB06 host cells

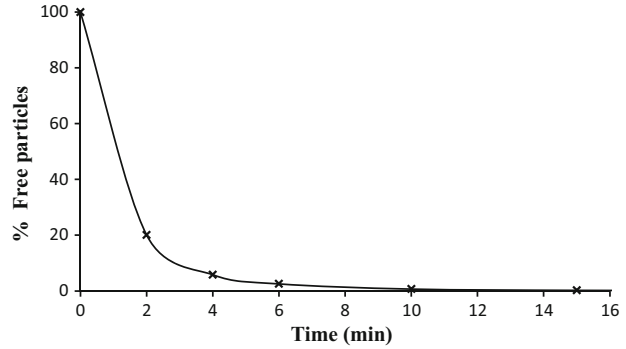

Fig. 2 Adsorption of bacteriophage MYSP06 to MYB06 cells. Approximately 80 % of the phage particles were adsorbed onto the cells at 2 min, and 99 % were adsorbed at 10 min post-infection. These experiments were repeated three times

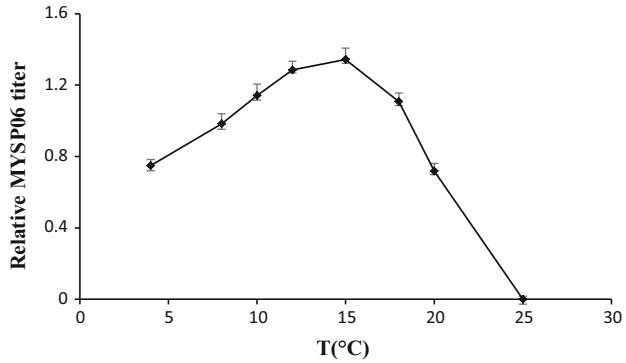

Fig. 3 Effect of temperature on phage production, as determined from the relative MYSP06 titer: The log P/P_0 values were plotted, with the value at P_0 representing the initial titer of MYSP06 and P representing the mean titer from triplicate assays after incubation for 6 h at different temperatures

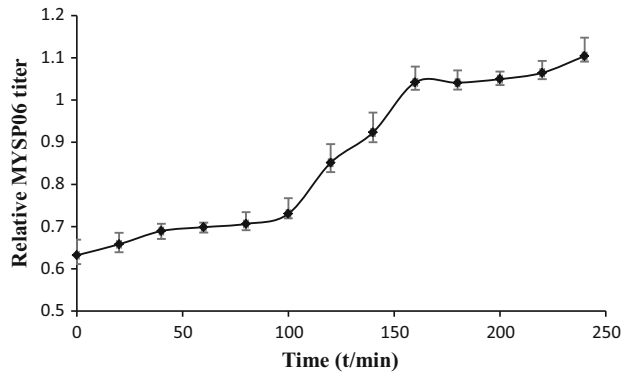

Fig. 4 One-step growth curve showing the relative MYSP06 titers: The log P/P_0 values were plotted, with P_0 representing the initial titer of infected MYB06 cells and P representing the mean titer from triplicate assays at time t

analysis (Fig. 8). At least 12 distinct protein bands, with molecular masses ranging from 15 to 100 kDa, were visualized after the gels had been stained using Coomassie

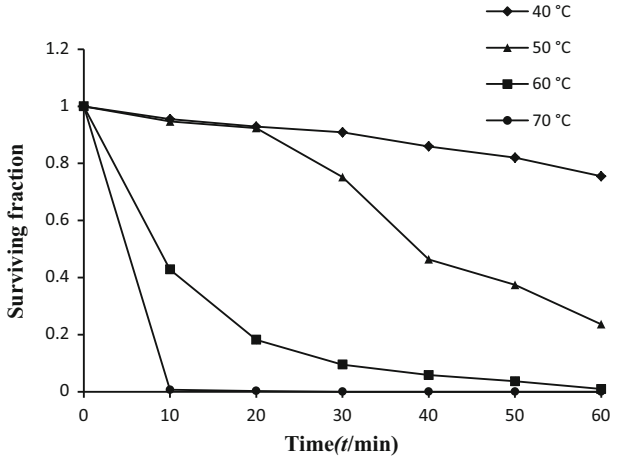

Fig. 5 Thermostability of the MYSP06 surviving fraction: The P/P_0 values were plotted, with P_0 representing the initial MYSP06 titer and P representing the mean titer from triplicate assays after incubation for 60 min at different temperatures

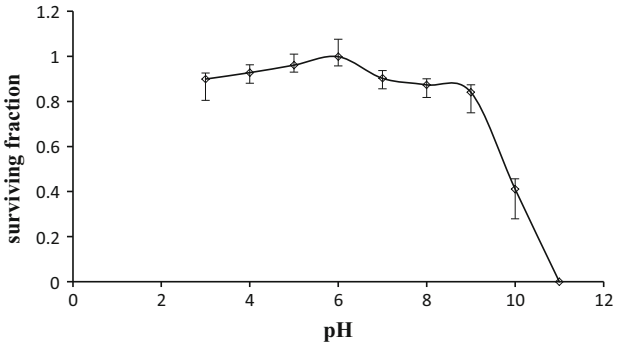

Fig. 6 The pH sensitivity of the surviving MYSP06 fraction: The P/P_0 values were plotted, with P_0 representing the initial MYSP06 titer and P representing the mean titer from triplicate assays after incubation for 1 h at different pH values

brilliant blue. The most abundant proteins were the 37 and 40 kDa proteins, which are most likely the major coat proteins of MYSP06.

Discussion

Microbes of the genus *Janthinobacterium* are aerobic, Gram-negative, soil-dwelling bacteria that have a distinctive dark-violet color. These organisms are of great practical industrial value due to their production of bacterial violacein. Among approximately 5500 known viruses, only 1 *Siphoviridae* bacteriophage has been observed to infect *Janthinobacterium* [2]. In this work, a new lytic cold-active bacteriophage, designated MYSP06, which possessed infective activity at 4 °C was specifically isolated from *Janthinobacterium* sp. MYB06 present in a water sample

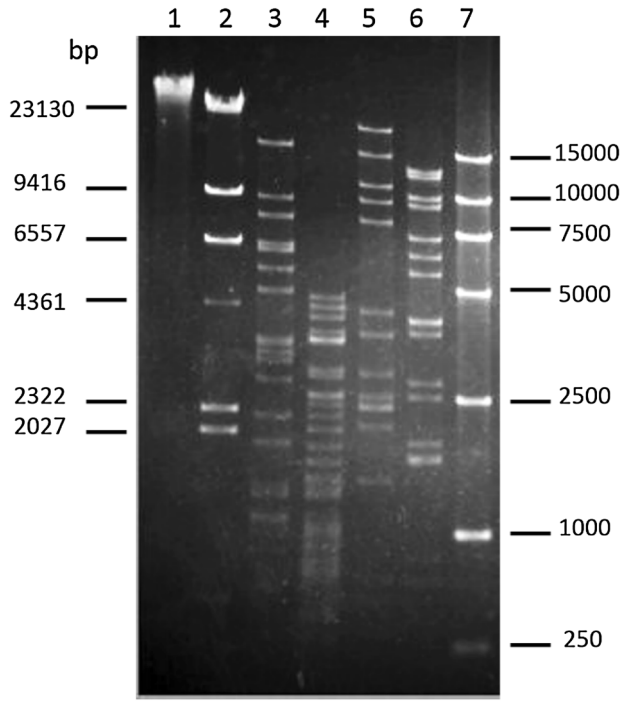

Fig. 7 The restriction endonuclease digestion patterns of MYSP06. *Lane 1* purified MYSP06 DNA, *Lane 2* molecular weight markers (bp), *Lane 3* MYSP06 DNA digested with *Eco*RI, *Lane 4* MYSP06 DNA digested with *Hin*dIII, *Lane 5* MYSP06 DNA digested with *Nde*I, *Lane 6* MYSP06 DNA digested with *Xba*I, *Lane 7* molecular weight markers (bp)

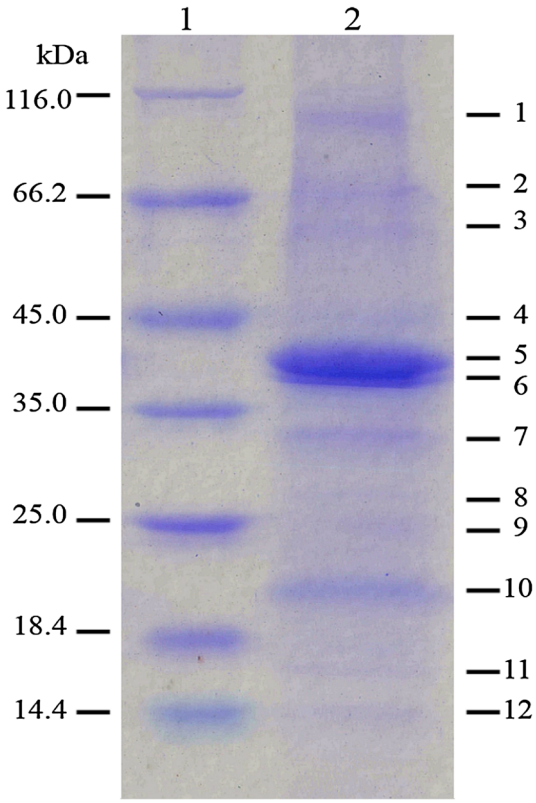

Fig. 8 SDS-PAGE of purified MYSP06. *Lane 1* protein markers (kDa). *Lane 2* purified MYSP06

collected from the Mingyong Glacier. To our knowledge, this is the first detailed study of a *Janthinobacterium* sp. virulent cold-active bacteriophage isolated from a glacier.

A tailed virus is the most abundant phage morphotype, and MYSP06 appeared to be affiliated with the family *Siphoviridae*; however, it is significantly different from the other known cold-active members of the family *Siphoviridae* (Table 1). Its tail was easily bent and was shorter than that of MYSP03 [15], and it was nearly three times the length of that of 11b [3]. Furthermore, phage MYSP06 formed plaques within a temperature range between 4 and 20 °C, which was wider than the range of 11b. The host cell *Janthinobacterium* sp. MYB06 is a psychrophilic bacterium with good growth at 30 °C, which is also higher than the optimal growth temperature of 11b, possibly due to their origins in different environments. MYSP06 was isolated from an inland plateau where the temperature is obviously different from day to night, leading to adaptation to a wide temperature range. However, 11b was isolated from the Arctic sea ice where the environment is permanently cold. It was assumed that their differential optimal growth temperatures may be the result of directional selection for survival in these environments. Moreover, the size of the genome of bacteriophage MYSP06 was much larger than that of 11b, and the genomes of the phages exhibited distinct restriction patterns after digestion with specific restriction endonucleases. This result may be related to differences in their shapes.

Moreover, the pattern of the proteins obtained from bacteriophage MYSP06 was different from those of 11b and MYSP03 [4, 15]. Although MYSP06 and 11b yielded multiple bands, their main bands were obviously different. Only four major bands (13, 32, 37 and 40 kDa) were obtained from MYSP03. The SDS-PAGE results suggested that MYSP06 is more similar to MYSP03 because they both yielded 37 and 39 kDa proteins; however, MYSP03 also contained a major protein of 14 kDa, which was also found in 11b. These results might reflect these bacteriophages having different natural environments and host specificities. The MYSP03 and 11b phages both yielded a band of 14 kDa, whereas the region from which MYSP03 and MYSP06 were derived may have led to their having similar bands of 37 and 39 kDa.

The basic strategy for understanding a bacteriophage is to study its biological characteristics. As a cold-active phage, MYSP06 was also sensitive to temperature. However, pH adaptation experiments revealed that MYSP06 was most stable in an acidic environment, whereas

Table 1 Characterization of siphoviruses MYSP06, 11b, and MYSP03

Phage	MYSP06	11b	MYSP03
Host	*Janthinobacterium* sp. MYB06	*Flavobacterium* sp. 11B	*Flavobacterium* sp. MYB03
Capsid (nm)	74	37–43	72
Tail length	210	75–91	240
Size of genome (kb)	65–70	30	66
Major protein bands (kDa)	Approximately 40, 37	Approximately 40, 16, 14	Approximately 40, 37, 32, 13
Growth temperature (°C)	4–20	0–10	4–20
Optimum growth (°C)	15		10

MYSP03 was stable in alkaline environments, and the survival pH range of MYSP06 was wider than that of MYSP03. The mechanism underlying this difference has not yet been defined. Moreover, in terms of sensitivity to chemical reagents, MYSP06 and MYSP03 were both insensitive to chloroform, showing that the capsid of both bacteriophages is not enveloped in lipids. Both bacteriophages were sensitive to SDS and to proteinase K but were insensitive to Triton X-100, which may reflect the similarity of their main structural proteins.

Despite its industrial value, *Janthinobacterium* causes soft-rot of cultivated button mushroom and is thus responsible for severe agricultural losses [16]. In the past, bactericides were widely used, which led to a dramatic increase in the number of resistant bacterial strains. Because strictly lytic bacteriophages can be used as antibacterial agents against bactericide-resistant bacterial strains [5], bacteriophage MYSP06 has a potential use in treating bacterial diseases in agricultural systems. With the development of bioinformatics and sequencing technologies, an increasing number of phage genomes have been sequenced, and a series of unique genes have been found. Further study and parsing of the MYSP06 genomic sequence will contribute to the understanding of different types of phages and their genetics under different geographical conditions and help to reveal the relationship between their abundance and edaphic and climatic factors.

Acknowledgments This study was supported by grants from the Scientific Research Program of the Higher Education Institution of XinJiang (Grant Number XJEDU2014S052), the Natural Science Foundation of China (30960079/31160121), and the China Scholarship Council. We are grateful to American Journal Experts (AJE) for critical reading of the manuscript.

References

1. Abedon ST (2009) Phage evolution and ecology. Adv Appl Mircobiol 67:1–45
2. Ackermann HW (2007) 5500 phages examined in the electron microscope. Arch Virol 152:227–243
3. Borriss M, Helmke E, Hanschke R, Schweder T (2003) Isolation and characterization of marine psychrophilic phage-host systems from Arctic sea ice. Extremophiles 7:377–384
4. Borriss M, Lombardot T, Glöckner FO, Becher D, Albrecht D, Schweder T (2007) Genome and proteome characterization of the psychrophilic *Flavobacterium* bacteriophage 11b. Extremophiles 11:95–104
5. Brüssow H (2012) What is needed for phage therapy to become a reality in Western medicine? Virology 434:138–142
6. Colisito DJ, Rogoff MH (1969) Characterization of lytic bacteriophages of *Bacillus thuringiensis*. J Gen Virol 5:267–274
7. Comeau AM, Chan AM, Suttle CA (2006) Genetic richness of vibriophages isolated in a coastal environment. Environ Microbiol 8:1164–1176
8. Danovaro R, Dell'Anno A, Corinaldesi C, Magagnini M, Noble R, Tamburini C, Weinbauer M (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. Nature 454:1084–1087
9. Desnues C, Rodriguez-Brito B, Rayhawk S, Kelley S, Tran T, Haynes M, Rohwer F et al (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature 452:340–343
10. Geslin C, Gaillard M, Flament D, Rouault K, Romencer ML, Prieur D, Erauso G (2007) Analysis of the first genome of a hyperthermophilic marine virus-like particle, PAV1, isolated from *Pyrococcus abyssi*. J Bacteriol 189:4510–4519
11. Holmfeldt K, Middelboe M, Nybroe O, Riemann L (2007) Large variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their *Flavobacterium* hosts. Appl Environ Microbiol 73:6730–6739
12. Kim JH, Gomez DK, Nakai T, Park SC (2010) Isolation and identification of bacteriophages infecting ayu *Plecoglossus* altivelis altivelis specific *Flavobacterium* psychrophilum. Vet Microbiol 140:109–115
13. LaFrentz BR, LaPatra SE, Call DR, Cain KD (2008) Isolation of rifampicin resistant *Flavobacterium* psychrophilum strains and their potential as live attenuated vaccine candidates. Vaccine 26:5582–5589
14. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–176
15. Li MY, Ji XL, Wang BQ, Zhang Q, Lin LB, Wei YL (2012) Isolation and characterization of a lytic bacterio from Mingyong glacier melt water. Acta Microbiol Sin 52:3354–3359
16. Lincoln SP, Fermor TR, Tindall BJ (1999) *Janthinobacterium agaricidamnosum* sp. nov., a soft rot pathogen of *Agaricus bisporus*. Int J Syst Bacteriol 49:1577–1589
17. Lin NT, Chiou PY, Chang KC, Chen LK, Lai MJ (2010) Isolation and characterization of ϕAB2: a novel bacteriophage of *Acinetobacter baumannii*. Res Microbiol 161:308–314

18. López-Bueno A, Tamames J, Velázquez D, Moya A, Quesada A, Alcamí A (2009) High diversity of the viral community from an Antarctic lake. Science 326:858–861
19. Lu Z, Breidt F, Fleming HP, Altermann E, Klaenhammer TR (2003) Isolation and characterization of a *Lactobacillus* plantarum bacteriophage, ΦJL-1, from a cucumber fermentation. Int J Food Microbiol 84:225–235
20. Mann NH, Cook A, Millard A, Bailey S, Clokie M (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424:741
21. Martha RJ, Clokie AMK (2008) Bacteriophages: methods and protocols, volume 1: isolation, characterization, and interaction. Humana Press, Totowa
22. Middelboe M, Nielsen TG, Bjørnsen PK (2002) Virus and bacterial production in the North Water: in situ measurements, batch-culture experiments and characterization and distribution of a virus–host system. Deep Sea Res PT II 49:5063–5079
23. Säwström C, Granéli W, Laybourn-Parry J, Anesio AM (2007) High viral infection rates in Antarctic and Arctic bacterioplankton. Environ Microbiol 9:250–255
24. Shen GH, Wang JL, Wen FS, Chang KN, Kuo CF et al (2012) Isolation and characterization of φkm18p, a novel lytic phage with therapeutic potential against extensively drug resistant *Acinetobacter baumannii*. PLoS One 7:1–8
25. Stenholm AR, Dalsgaard I, Middelboe M (2008) Isolation and characterization of bacteriophages infecting the fish pathogen *Flavobacterium* psychrophilum. Appl Environ Microbiol 17: 4070–4078
26. Su MT, Venkatesh TV, Bodmer R (1998) Large-and small-scale preparation of bacteriophage lambda lysate and DNA. Biotechniques 25:44–46
27. Wang F, Wang F, Li Q, Xiao X (2007) A novel filamentous phage from the deep-sea bacterium *Shewanella piezotolerans* WP3 is induced at low temperature. J Bacteriol 189:7151–7153
28. Weiss BD, Capage MA, Kessel M, Benson SA (1994) Isolation and characterization of a generalized transducing phage for *Xanthomonas campestris* pv. *campestris*. J Bacteriol 176: 3354–3359
29. Wells LE, Deming JW (2006) Characterization of a cold-active bacteriophage on two psychrophilic marine hosts. Aquat Microb Ecol 45:15–29
30. Williamson SJ, Rusch DB, Yooseph S, HalpernAL Heidelberg KB et al (2008) The Sorcerer II Global Ocean Sampling Expedition: metagenomic characterization of viruses within aquatic microbial samples. PLoS One 3:1456–1469
31. Yang Q, Zheng D (1990) On altitudinal land use zonation of the Hengduan Mountain region in southwestern China. GeoJournal 20:369–374
32. Ye T, Zhang X (2008) Characterization of a lysin from deep-sea thermophilic bacteriophage GVE2. Appl Microbiol Biotechnol 78:635–641