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ORIGINAL PAPER

Genome sequence of temperate bacteriophage Psymv2 from Antarctic Dry Valley soil isolate *Psychrobacter* sp. MV2

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Abstract A temperate phage, Psymv2, was isolated from an Antarctic soil bacterium, *Psychrobacter* sp. MV2. The morphology of Psymv2 was typical of the *Siphoviridae*, with an isometric head and non-contractile tail. The Psymv2 genome was found to be 35,725 bp in length, had a G + C content of 44.5 %, with 49 protein-coding genes and one tRNA gene predicted. Integration of Psymv2 occurred at an *ssrA* gene, with the last 27 bases of this gene directly repeated at the prophage ends. The genome was organised in a modular fashion: integration, regulation, packaging, head assembly, tail assembly, host specificity and lysis. While the genome sequence had little similarity on a nucleotide level to previously reported phage sequences, the genome architecture resembled that of *Siphoviridae* of low G + C Gram-positive bacteria. The closest relatives to Psymv2 were uncharacterized putative prophages within the *P. arcticus* 273-4 and *Acinetobacter baumannii* 6013113 genomes. Global alignment of the Psymv2 genome and these prophages revealed significant conservation of the structural modules despite the large spatial divergence of their hosts. A number of unique ORFs were identified in the Psymv2 genome that may contribute to phage and lysogen fitness.

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Abbreviations

aa	Amino acid
CDS	Coding sequence
LCB	Local collinear block
MC	Mitomycin C
ORF	Open reading frame
TMD	Transmembrane domain

Introduction

The McMurdo Dry Valleys of eastern Antarctica are one of the most extreme environments on earth; characterised by low temperatures (fluctuating from −55 °C to +25 °C), low water and nutrient availability, high soil salinity, high radiation and strong katabatic winds (Doran et al. 2002; Horowitz et al. 1972; Vincent 1988). These cold desert soils harbour a surprisingly diverse range of microbes, the majority of which are taxonomically distinct and, as yet, uncultured (Pointing et al. 2009; Smith et al. 2006). Microbial activity in this extreme environment is thought to be largely shaped by abiotic factors (Cary et al. 2010). Allen et al. (2010) have, however, recently shown that phages play a crucial role in the regulation of microbial community structure in Arctic soils.

While aquatic Antarctic viral communities are known to be highly diverse (Lisle and Priscu 2004; López-Bueno et al. 2009; Säwström et al. 2008) the abundance and impact of viruses in Antarctic soils has received little to no research attention. In one of the few studies to date, soils collected from the rims of Antarctic ephemeral ponds were

shown to contain only 16-fold less extracellular virus like particles than moist organic rich soils (Williamson et al. 2007). The incidence of lysogeny within these soils was additionally estimated by Williamson et al. (2007) and a correlation between water content and lysogeny observed; with a potential increase in lysogeny in drier soils.

The cultivation and genomic characterisation of cold-adapted viruses from this unique environment is of great interest as these organisms potentially represent important elements of the Antarctic desert soil trophic structure, and may play key roles in critical processes such as lateral gene transfer. In this study we screened cultured psychrophilic Dry Valley soil isolates for prophages and report on the isolation of phage Psymv2 from a *Psychrobacter* spp. To our knowledge this is the first report of the isolation and complete genome sequencing of a bacteriophage specific for a cold-adapted terrestrial microbe.

Materials and methods

Sampling, enrichment and isolation of bacterial strains

Soil samples were collected aseptically from beneath hypoliths in Miers Valley (78°05′S, 163°45′E) in the McMurdo Dry Valleys, South Victoria Land, Antarctica. Samples were stored at −80 °C and defrosted at 4 °C prior to use. Bacterial enrichment cultures were established by suspending soil samples (ca. 1–1.5 g) in 6 ml half-strength liquid Nutrient Broth (Merck) and incubated at 16 °C for 2.5 h with intermittent shaking. Dilution series of the enrichment cultures were plated onto half-strength Nutrient Agar (Merck) and isolates grown at 16 °C for 2–7 days. Isolated colonies were picked and subjected to additional rounds of purification by inoculation onto fresh NA plates. Isolates capable of growth at 4 °C were selected for induction experiments.

The 16S rRNA gene was amplified by PCR from genomic DNA using the universal bacterial primers E9F and U1510R (Hansen et al. 1998; Reysenbach and Pace 1995). The PCR product was cloned using the pGem-T plasmid cloning system (Promega) according to standard procedures. Plasmids were sequenced with M13 primers using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit on an ABI PRISM 3130xl Genetic Analyzer (Perkin Elmer). Sequence identity was analysed by comparison to the GenBank nr/nt database using the BLASTN program (Altschul et al. 1990).

Prophage induction and purification

Isolates were screened for prophages through the addition of various concentrations (0.001–6 μg/ml) of Mitomycin C (MC; Sigma–Aldrich) to mid-log phase cultures for 1 h at 16 °C. Bacterial cells and debris were removed from induced cultures by low speed centrifugation and filter sterilisation using 0.45 μm pore size syringe filters (Nalgene). Bacteriophage suspensions for electron microscopy analyses and DNA isolation were prepared using the method of Ackermann (2009). Briefly, bacteriophages were collected from filtered suspensions by centrifugation at 25,000 *g* for 60 min in a fixed-angle rotor. Phages were then washed at least twice with 0.1 M ammonium acetate (pH 7) and sedimented at each wash step by centrifugation as described. Sedimented phages were gently resuspended in 0.1 M ammonium acetate (pH 7).

Transmission electron microscopy

For negative staining, bacteriophage suspensions were spotted onto carbon-coated copper grids for 1 min, stained for a further minute with 2 % (wt/vol) uranyl acetate, pH 4 and air-dried (Ackermann 2009). Phages were visualised using a Jeol 200CX electron microscope, at 60 kV and magnifications ranging from 50,000× to 100,000×.

Phage DNA isolation

Viral genomic DNA was extracted using a standard proteinase K and SDS protocol (Sambrook and Russell 2001). Briefly, phage preparations were incubated with both DNase (5 mg/ml) and RNase (10 mg/ml) for 2 h at 37 °C. Samples were then treated with Proteinase K (100 μg/ml) in the presence of 0.5 % SDS for 1 h at 65 °C, extracted with phenol/chloroform, and the DNA recovered from the aqueous phase by ethanol precipitation.

Cloning, sequencing and analysis

Genomic DNA from *Psychrobacter* sp. MV2 was cloned into the pCC1FOS™ vector (Epicentre) using the manufacturer's recommended protocol. Fosmid clones harbouring the phage genome were identified by Southern blot using Psymv2 DNA, labelled with the DIG-DNA labelling kit (Roche), as probe. Four fosmid clones, with an average insert size of approximately 30 kb, were sequenced by Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, SA) using a 454 GS-FLX platform (Roche Diagnostics). The genome sequencing of phage Psymv2 was completed by PCR amplification directly from genomic DNA. The chromosomal insertion site of the bacteriophage was sequenced by genome walking from an adaptor ligated *Psychrobacter* sp. MV2 genomic library using the method of Siebert et al. (1995).

Sequence analysis and assembly was carried out using the CLC Genomics Workbench package (CLC bio, Katrinebjerg, Denmark). The accuracy of the genome sequence

assembly was verified by amplification by PCR. Ab initio gene predictions were performed using GeneMark.hmm 2.0 (http://www.exon.biology.gatech.edu/heuristic_hmm2.cgi) (Besemer and Borodovsky 1999), fgenesv and fgenesb (http://www.linux1.softberry.com/berry.phtml) (Softberry, Mount Kisco, NY, USA) using the generic bacterial genetic code. tRNA and tmRNA genes were predicted using Aragorn (http://www.130.235.46.10/ARAGORN/) (Laslett and Canback 2004) and BRUCE v1.0 (Laslett et al. 2002). Sequences were compared to the GenBank_nr database using the Blastp and/or tblastx algorithms with an *E*-value cut-off of 10^{-5}. Annotations were additionally based on results from RPS-BLAST searches, with an *E*-value threshold of 10^{-2}, against the NCBI conserved domains database or CDD (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler-Bauer et al. 2009) and Interpro scan searches (http://www.ebi.ac.uk/Tools/InterProScan/) (Hunter et al. 2009). Transmembrane domains were predicted using the Transmembrane hidden Markov model TMHMM v2.0 (Krogh et al. 2001) and protein localisation predicted using PSORTb v3.0.2 (Yu et al. 2010). Genome alignments with the closest prophages were performed using the progressive Mauve algorithm (Darling et al. 2010).

Nucleotide sequence accession numbers

The 16S rRNA (1,496 bp) and *ssrA* gene sequences of *Psychrobacter* sp. MV2 and the phage Psymv2 genome were deposited in GenBank under accession numbers HQ610925, JF270479 and JF270478, respectively.

Results and discussion

Isolation and morphology of bacteriophage Psymv2

Twenty-two psychrophilic microbial strains were cultured from soil collected from the Miers Valley, Antarctica, and screened for prophages by treatment with Mitomycin C (MC). Phage Psymv2 was induced from an aerobic non-motile Gram-negative coccobacillus, with optimal induction achieved at 1 μg/ml MC. The 16S rRNA gene sequence of the bacterial isolate had 99.06 % identity to that of the strict psychrophile *Psychrobacter frigidicola* type strain DSM 12411T (Bowman et al. 1996; GenBank accession number AJ609556). The isolate, termed *Psychrobacter* sp. MV2, had an optimal growth temperature of 16 °C and was incapable of growth above 27 °C. Members of the genus *Psychrobacter*, within the class *Gammaproteobacteria*, have been isolated from a wide variety of low temperature marine and terrestrial environments, as well as various other sources such as food products, human tissues and body fluids. The widespread distribution of members of this genus reflects their capacity for reproduction within a wide range of temperatures (−10 to 42 °C) (Bowman et al. 1996; Maruyama et al. 2000; Romanenko et al. 2004; Bowman 2006; Bakerman et al. 2006). Several psychrotrophic strains, such as the recently sequenced *Psychrobacter arcticus* 273-4 (Ayala-del-Río et al. 2010) isolated from Siberian permafrost, are seen as models for understanding cold-adaptation in microorganisms. No further characterisation of *Psychrobacter* sp. MV2 has been carried out to date.

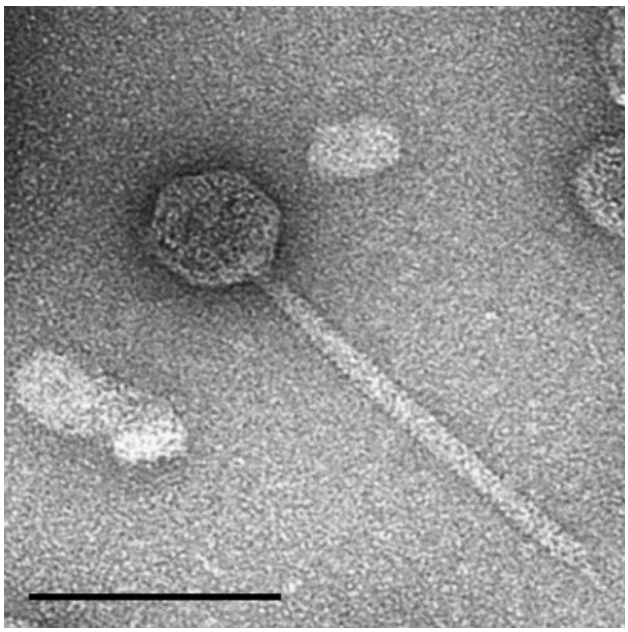

Fig. 1 Transmission electron micrograph of *Psychrobacter* phage Psymv2. Phages were negatively stained with 2 % uranyl acetate. The *scale bar* represents 100 nm

The morphology of Psymv2 was examined by electron microscopic analysis of phage particles (Fig. 1). Psymv2 had an isometric head with an average diameter of approximately 53 nm and an approximately 188 nm long non-contractile tail. Terminal tail fibres were additionally visible. This is the characteristic morphology of the B1 morphotype group of the *Siphoviridae* family, order *Caudovirales* (Ackermann 2007).

General features of the Psymv2 genome

The Psymv2 genome was sequenced and annotated as outlined in the Materials and Methods. The double stranded (ds) DNA genome was 35,725 base pairs (bp) in length, with an average guanine-plus-cytosine (G + C) content of 44.5 %. This is similar to the G + C composition of the currently sequenced *Psychrobacter* strains: *P. arcticus* 273-4 (42.8 %; Ayala-del-Río et al. 2010), *P. cryohalolentis* strain K5 (42 %; Genbank NC_007969) and

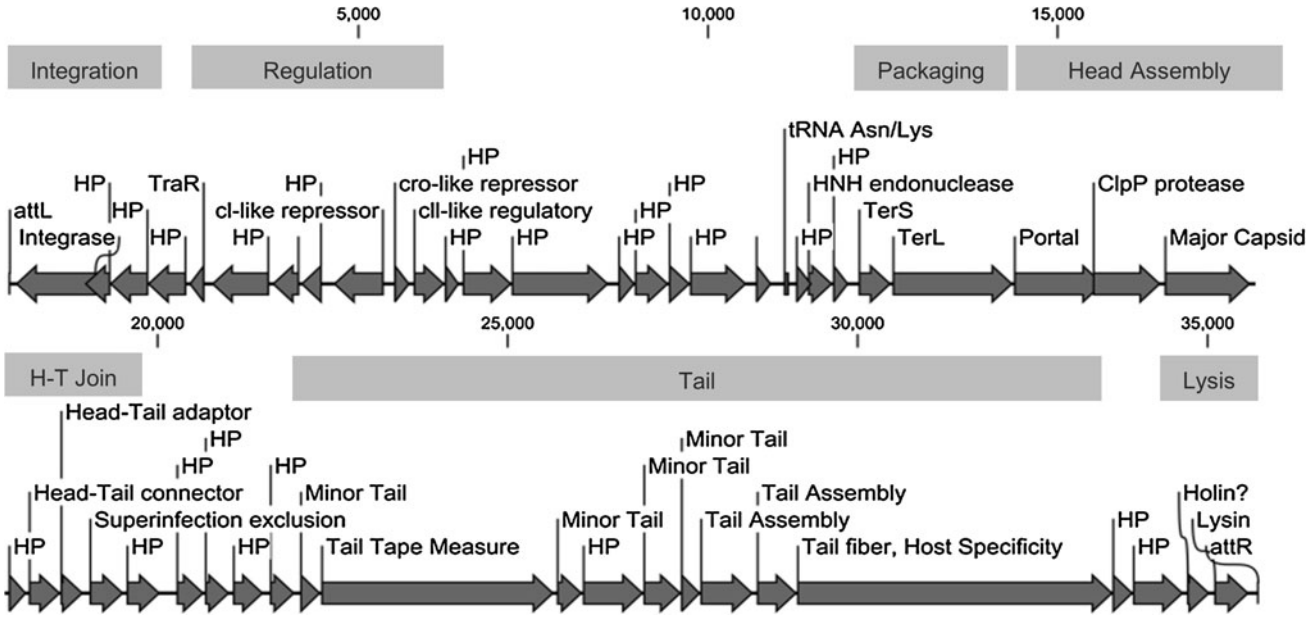

Fig. 2 Schematic illustration of phage Psymv2 in the *Psychrobacter* sp. MV2 genome. The nucleotide sequence numbering is shown above and predicted open reading frames (ORFs) illustrated by *arrows* indicating the direction of transcription. ORFs with functional annotations are labelled, the abbreviations terS and terL are for the small and large terminase subunits respectively. *HP* indicates hypothetical proteins and *H-T*, head-to-tail. The location of the phage *att* sites are shown

Psychrobacter sp. PRwf1 (44 %; Genbank NC_009524). Segments within the phage genome with lower G + C contents, that may delineate functional clusters and/or regions of lateral gene transfer, were identified and are discussed later.

A total of 49 putative open reading frames (ORFs), with a minimum of 50 amino acids (aa) coding length, were identified. The start codon usage for the ORFs was 75.5 % ATG (37/49) and 24.5 % GTG (12/49) and nine of these ORFs are transcribed from the complementary strand. The deduced amino acid sequence of each ORF was compared to the NCBI nr database using Blastp software with an *E*-value cut-off of e-5. Of the 49 Psymv2 coding sequences (CDS), 34 (71.4 %) had homologues in the Genbank database, with top hits to proteins encoded by uncharacterised functional or cryptic prophages (76.5 % or 26/34), characterised phages (11.7 % or 4/34) and bacterial genomes (11.7 % or 4/34). The latter may, however, be present in as yet unrecognised prophage and prophage-like elements. Predicted functions could be assigned to 51 % (25/49) of the phage Psymv2 proteins, based on sequence homology, conserved motifs, HMM profiles and other sequence signatures. The remaining 24 (49 %) protein products could not be assigned a putative function. A single tRNA gene specific for Lys/Asn at bp 11,082–11,160 was predicted with Aragorn (Laslett and Canback 2004). The genetic map of the Psymv2 prophage is given in Fig. 2. The positions, putative functions and top Blastp hits of the proteins encoded by Psymv2 are given in Table 1.

The genome of phage Psymv2 displayed a compact modular arrangement of functional gene clusters for integration, regulation, packaging, head morphogenesis, head–tail joining, tail morphogenesis and lysis (Fig. 2). While the order of functional modules is similar to that of the Lambda-like group of *Siphoviridae* phages (reviewed by Casjens 2005), the position of the genes responsible for host cell lysis downstream of the tail morphogenesis gene cluster is atypical. A similar arrangement has been noted in other *Siphoviridae* phages (Casjens 2008), including the well-studied *Pseudomonas* phage D3 (Kropinski 2000), the recently described *Burkholderia* phage phi644-2 (Ronning et al. 2010) and several phages infecting low G + C Gram-positive bacteria (Canchaya et al. 2003).

The closest related elements to Psymv2, based on the greatest number of shared homologous proteins, were uncharacterised prophages within the genomes of members of the *Pseudomonadales*. An uncharacterised lambda-like prophage between bp 551715 and 585095 in the *P. arcticus* 273-4 genome (Genbank accession number: NC_007204; Ayala-del-Río et al. 2010) shared the greatest number of homologous proteins with the highest identity (35–93 %). These clustered within the tail morphogenesis modules of both prophages. Proteins within the packaging and head morphogenesis module of Psymv2 had the highest identity (62–74 %) to proteins encoded by an uncharacterised prophage in the whole genome shotgun sequence of *Acinetobacter baumannii* 6013113 (Genbank accession number: NZ_ACYR01000154.1). For the purposes of this paper

Table 1 Putative ORFs within the *Psychrobacter* phage Psymv2 genome

ORF	Range (bp)	Size (aa)	Predicted function	Bacterial strain/phage with top BLASTP hit (accession; *E* value)	% Identity; % similarity (query coverage)	Conserved domains database hit(/s) with $E < 0.001$ (accession); TMHMM predicted transmembrane domain(/s) or TMD(/s)
1	1,251–97	384	Integrase	*Acinetobacter* sp. 6,013,113 (ZP_06784515; 6e-151)	66; 81 (99)	DNA_BRE_C super family (cl00213)
2	1,454–1,086	122	Unknown	Acinetobacter baumannii SDF (YP_001706828; 2e-09)	43; 58 (86)	None
3	1,995–1,444	183	Unknown	None	–	None
4	2,539–1,985	184	Unknown	*Psychrobacter arcticus* 273-4 (YP_264266; 7e-90)	85; 92 (99)	None
5	2,799–2,578	73	Transcriptional regulator	*Psychrobacter arcticus* 273-4 (YP_263775; 3e-13)	59; 72 (86)	zf-dskA_traR super family (cl00755)
6	3,723–2,902	273	Unknown	*Pseudomonas* syringae *pv. tomato* T1 (ZP_03396200; 1e-31)	36; 56 (91)	DUF2303 super family (cl02338)
7	4,154–3,768	128	Unknown	*Pseudomonas syringae pv.* B728a (YP_235914;9e-17)	43; 58 (85)	None
8	4,477–4,178	99	Unknown	None	–	None; 1 TMD
9	5,363–4,644	239	CI-like repressor protein	*Pseudomonas* phage D3 (NP_061565; 5e-20)	32; 50 (96)	S24_LexA-like (cd06529); HTH_XRE (cd00093)
10	5,509–5,733	74	Cro-like repressor	*Pseudomonas* phage D3 (NP_061566; 1e-09)	50; 67 (81)	COG4197 super family (cl01714)
11	5,788–6,231	147	Regulatory protein CII-like	*Burkholderia* phage Bcep176 (YP_355349; 3e-16)	38; 58 (92)	Phage_CP76 super family (cl06105)
12	6,234–6,440	68	Unknown	None	–	None
13	6,489–7,190	233	DNA-binding protein (Roi)	*Burkholderia thailandensis* TXDOH (ZP_02373126; 2e-48)	49; 63 (91)	ANT super family (cl01462); Phage_pRha super family (cl110713)
14	7,190–8,575	461	Unknown	*None*	–	DUF1376 super family (cl01531)
15	8,715–8,954	79	Unknown	None	–	None
16	8,951–9,433	160	Unknown	*Acinetobacter baumannii* SDF (YP_001707727; 2e-24)	42; 61 (86)	None
17	9,438–9,743	101	Unknown	None	–	None
18	9,740–10,549	269	Unknown	*Psychrobacter arcticus* 273-4 (YP_264282.1; 4e-48)	51; 71 (68)	None
19	10,683–10,907	74	Unknown	None	–	None
20	11,082–11,160	–	tRNA-Lys/Asn	–	–	–
20	11,257–11,484	75	Unknown	None	–	None
21	11,427–11,780	117	HNH endonuclease	*Acinetobacter* sp. 6,013,113 (ZP_06784485; 8e-34)	64; 74 (94)	HNHc (cd00085)
22	11,786–12,010	74	Unknown	None	–	None
23	12,152–12,616	154	Terminase small subunit	*Acinetobacter* sp. 6,013,113 (ZP_06784483; 3e-53)	62; 77 (100)	Terminase_4 super family, P27 family (cl01525)
24	12,639–14,357	572	Terminase large subunit	*Acinetobacter* sp. 6,013,113 (ZP_06784482; 0.0)	74; 86 (99)	Terminase_1 super family (cl10609)
25	14,375–15,655	426	Portal	*Acinetobacter* sp. 6,013,113 (ZP_06784481; 2e-170)	72; 84 (96)	Phage_portal super family (cl01923)
26	15,504–16,472	322	ClpP protease	*Acinetobacter* sp. 6,013,113 (ZP_06784480; 3e-109)	68; 84 (85)	S14_ClpP_1 (cd07016)
27	16,530–17,756	408	Major capsid protein	*Acinetobacter* sp. 6,013,113 (ZP_06784479; 1e-151)	68; 80 (97)	Phage_capsid super family (cl12304)
28	17,870–18,124	84	Unknown	None	–	None

Table 1 continued

ORF	Range (bp)	Size (aa)	Predicted function	Bacterial strain/phage with top BLASTP hit (accession; *E* value)	% Identity; % similarity (query coverage)	Conserved domains database hit(/s) with $E < 0.001$ (accession); TMHMM predicted transmembrane domain(/s) or TMD(/s)
29	18,158–18,610	150	Head completion	*Pseudomonas putida* W619 (YP_001748215; 3e-19)	40; 62 (100)	HK97 gp6 (cd08054)
30	18,612–18,935	107	Head-to-tail adaptor	*Pseudomonas aeruginosa* 2,192 (ZP_04938423; 5e-18)	41; 65 (97)	Phage_H_T_join super family (cl11461)
31	19,026–19,511	161	Superinfection exclusion	*RhodoPseudomonas palustris* HaA2 (YP_486987; 4e-34)	68; 80 (65)	Lipoprotein_Ltp super family (cl06542), 1 TMD
32	19,555–20,034	159	Unknown	*Psychrobacter arcticus* 273-4 (YP_264863; 3e-40)	84; 94 (57)	None, 1 TMD
33	20,269–20,661	130	Unknown	*Psychrobacter arcticus* 273-4 (YP_263755; 6e-15)	35; 51 (96)	DUF646 super family (cl12124)
34	20,675–21,025	116	Unknown	*Acinetobacter radioresistans* SK82 (ZP_05362067;1e-10)	45; 62 (66)	DUF3168 super family (cl12902)
35	21,075–21,509	144	Unknown	None	–	None
36	21,602–21,958	118	Unknown	None	–	None
37	22,036–22,326	96	Minor tail protein	None	–	Phage_tail_T super family (cl05636)
38	22,336–25,659	1,107	Tail tape measure protein	*Moraxella catarrhalis* RH4 (YP_003627118; 1e-156)	33; 50 (96)	tape_meas_TP901 (TIGR01760), 4 TMDs
39	25,702–26,064	120	Minor tail protein	*Psychrobacter arcticus* 273-4 (YP_263747; 1e-54)	93; 96 (90)	Phage_min_tail super family (cl01940)
40	26,074–26,937	287	Unknown	*Acinetobacter baumannii* AB900 (ZP_04662675; 8e-52)	43; 76 (99)	None
41	26,939–27,478	179	Minor tail protein	*Psychrobacter arcticus* 273-4 (YP_263743; 7e-62)	66; 76 (99)	Phage_tail_L super family (cl01908)
42	27,475–27,753	92	Minor tail protein	*Psychrobacter arcticus* 273-4 (YP_263743; 6e-41)	90; 95 (97)	Phage_tail_L super family (cl01908)
43	27,753–28,505	250	Tail assembly protein	*Psychrobacter arcticus* 273-4 (YP_263742; 4e-115)	78; 90 (100)	MPN_NLPC_P60(cd08073);Spr cell wall-associated hydrolases (COG0791)
44	28,559–29,131	190	Tail assembly protein	*Psychrobacter arcticus* 273-4 (YP_263741; 2e-78)	87; 90 (100)	Lambda_tail_I super family (cl01945); 2 TMDs
45	29,134–33,645	1,503	Tail fibre, host specificity	*Psychrobacter arcticus* 273-4 (YP_263740; 0.0)	84; 89 (59)	Phage-related protein, tail component (COG4733)
46	33,639–33,932	97	Unknown	None	–	None
47	33,932–34,651	239	Unknown	None	–	None
48	34,702–35,028	108	Holin	Psychrobacter arcticus 273-4 (YP_263738; 2e-50)	93; 97 (100)	None, 2 TMDs
49	35,094–35,588	164	Lysin	Bordetella phage BPP-1 (NP_958672; 2e-45)	56; 71 (97)	DUF847 super family (cl09583); PG_binding super family (cl09627)

these putative prophages will be termed Psyar273-4/1 and Acba6013113/1, respectively.

Host interaction and phage regulation modules

The product of Psymv2 ORF1 is a putative integrase protein with conserved motifs of the tyrosine site-specific recombinase family. The Psymv2 integrase had 65 % identity to the predicted integrase encoded by Acba 6013113/1 and 41 % to that encoded by Psyar273-4/1. The integrase had 25–30 % identity to numerous phage integrase proteins, with the highest identity (30 %) to the integrase (Bbp50) of the podovirus *Bordetella* BPP-1. Several proteins involved in phage regulation were identified, with Psymv2 ORFs 9, 10 and 11 encoding lambda CI-, Cro- and CII-like proteins, respectively. The CI and Cro repressor proteins had the highest identity to the cognate proteins of *Pseudomonas* phage D3 (32 and 50 % identity, respectively), while the CII homolog had 38 % identity to gp14 of *Burkholderia* phage Bcep176. None of

these proteins showed any sequence identity to those ascribed the same function in Psyar273-4/1 or Acba6013113/1. The regulatory region additionally contains a DNA-binding protein (encoded by ORF 13) that may function in a manner similar to the Roi protein, providing superinfection immunity.

In the majority of characterised dsDNA bacteriophages, host cell lysis is a result of the combined action of two proteins; endolysin and holin (Wang et al. 2000). Typical bacteriophage endolysins have an N-terminal catalytic domain and a C-terminal cell wall binding domain. While the protein encoded by Psymv2 ORF 49 did not show significant similarity to characterised bacteriophage endolysins, the presence of these two conserved domains within the protein facilitated functional assignment. The product of ORF 49 is a 164 aa polypeptide with an N-terminal domain (aa 6–88) that shows significant sequence similarity (*E*-value 5.85e-20) to DUF847 super family members, predicted to be related to lysozyme enzymes. The C-terminal region (aa 91–154) contains a potential peptidoglycan binding domain (*E*-value 2.17e-15) (Table 1). Interestingly, the closest Blastp match to the Psymv2 putative lysin was to the *Bordetella* phage BPP-1Bbp2 protein (56 % identity) of the *Podoviridae* family (Table 1).

Holins represent one of the most diverse groups of proteins, with greater than 50 divergent gene families. The lack of sequence similarity between members of this group is probably a reflection of the evolutionary pressures related to their function as regulators of the duration of the lytic infection cycle (Wang et al. 2000). Holins are typically small proteins of between 71 and 161 aa, with two to three transmembrane domains, and may possess a dual translation initiation site. In most phages the ORF encoding holin immediately precedes or overlaps the endolysin gene (Wang et al. 2000). Psymv2 ORF 48, immediately upstream of the ORF encoding the predicted Psymv2 lysin, encodes a small protein of 108 aa. This protein contains two predicted transmembrane domains (TMDs) (aa 10–32 and aa 45–67) and may be localised to the cytoplasmic membrane (PSORTb localisation score 10.00). The first two codons of ORF48 are the initiation codons GTG and ATG. Therefore, this protein may function as a class II holin.

Packaging and structural modules in Psymv2

The packaging module of phage Psymv2 contains ORFs 23 and 24, which are predicted to encode the small and large subunits of the phage terminase, respectively (Table 1). The small subunit is responsible for recognising and binding the *cos* site in concatemeric phage DNA, the endonuclease and ATPase activities of the large subunit are then responsible for cleaving the DNA for packaging into the phage head (Rao and Feiss 2008). Based on sequence similarity and the presence of conserved domains, ORFs 25, 26 and 27 are predicted to encode the portal protein, the phage maturation protease and the major capsid protein, respectively (Table 1). The packaging and head morphogenesis proteins of phage Psymv2 have 62–68 % identity to related proteins of Acba6013113/1 and show little to no identity to those encoded by Psyar273-4/1. The Psymv2 portal, protease and capsid show homology to cognate proteins of the *Enterobacteria* phage HK97 and *Pseudomonas* phage D3.

In the majority of *Siphoviridae* the packaging and head genes are followed by a cluster of head or tail completion and tail encoding genes. A similar arrangement was seen in the Psymv2 genome (Fig. 2; Table 1). Psymv2 ORFs 29 and 30, with overlapping stop and start codons, encode proteins that share conserved motifs found in the head (or tail) completion proteins of long-tailed phages such as HK97 (Cardarelli et al. 2010). Head (or tail) completion proteins are specialised proteins of the phage head that serve as an attachment point for the tail and allow for the movement of the DNA out of the capsid during infection.

The tail morphogenesis module of Psymv2 spans ORFs 37–45. A number of putative minor tail components and tail assembly proteins are encoded by ORFs 37, 39, 41-44. The protein encoded by ORF 38 had only limited homology to any known or hypothetical phage proteins but was identified as the phage tape measure protein (tmp) based on the presence of four predicted TMDs (aa 391–413, 437–459, 519–541 and 548–570), and a conserved core region of a family of phage tail proteins, which includes the TP901 tmp. Additionally, the size of the encoded protein (1,107 aa) corresponds well with the measured Psymv2 tail length of approximately 188 nm. It is well established that the tmp determines tail length (Katsura and Hendrix 1984). Psymv2 ORF 43 encodes a tail-associated protein with an N-terminal metallo-endopeptidase motif (aa 6–109) and a C-terminal domain (aa 124–226) with predicted cell-wall hydrolase activity. This tail-associated protein may then function during cell adsorption and penetration as a peptidoglycan-degrading enzyme. The enzymatic activity of the protein would cleave both the peptide cross-links and polysaccharide chains within the peptidoglycan cell wall, facilitating entry of the dsDNA genome through the phage tail and into the host cell. Psymv2 ORF45 encodes a large protein of 1,503 aa, the N-terminal region (aa 1–896) showed significant sequence identity (84 %, *E*-value 0.0) to the cognate protein of Psyar273-4/1. This protein has similarity to the tail fibres of a number of lambda-like phages, including HK97 and HK022, and is the putative phage tail fibre involved in determining host specificity.

Embedded within the Psymv2 structural cluster were a number ORFs encoding proteins of unknown function but conserved sequence, including ORF 33 and 34, which encode members of the DUF646 and DUF3168 families, respectively (Table 1). ORFs 31 and 32 may be involved in superinfection exclusion, and are discussed later.

Comparison of Psymv2 with related phages: identification of unique regions

A global genome alignment of Psymv2 with the closest known elements Psyar273-4/1 and Acba6013113/1 revealed the mosaic arrangement of the functional modules within the Psymv2 genome (Fig. 3a). Clearly defined regions of nucleotide sequence homology or local collinear blocks (LCB), indicated by similarly coloured blocks in Fig. 3a, were apparent. The first such region spans the ORF encoding the integrase in all three phages. The packaging and head module (ORF 21–30) of Psymv2 showed clear homology to the same region in Acba6013113/1. Part of the Psymv2 tail morphogenesis module (ORFs 39–45), including the host specificity protein, showed homology to the module within the Psyar273-4/1 genome. The abrupt cessation of the homologous regions between the phages suggests modular exchanges or rearrangements and insertions. These results support the current view that the genomes of many tailed phages are mosaics that arise through genetic exchange from a diverse pool of phages (Casjens 2005). While large sections of the three phage genomes share little nucleotide sequence similarity, the overall order of functional modules is conserved (Fig. 3a). As if often the case, this is a reflection of the conserved regulatory and transcriptional control patterns governing phage life cycles.

To gain a greater understanding of the homology of Psymv2 to other bacteriophages, and to identify proteins that are unique to Psymv2, the Psymv2 predicted proteins were compared to both the NCBI nr database and proteins of viral origin using Blastp (Fig. 3b, c, respectively). From this it was clear that both the head and tail structural proteins of Psymv2 show the highest levels of homology to cognate proteins in prophages (Fig. 3b) but also have detectable homologues in a number of *Siphoviridae* phages, including the well-studied HK97, HK022 and D3 (Fig. 3c). The structural proteins, particularly the terminase and procapsid, are often the most conserved proteins of tailed phages (Casjens 2008). The notable exception within the structural morphogenesis region was the tmp (Box in Fig. 3b, c). While no single phage shared homologues to all or even a majority of the proteins involved in integration, regulation and lysis, detectable homologues were identified amongst a number of phages in the *Siphoviridae*, *Podoviridae* and *Myoviridae* families (Fig. 3c).

The Psymv2 genome contains four regions that appear to encode proteins specific to Psymv2 with no homologues encoded by known phages and with lower G + C contents (Grey shading in Fig. 3b, c). The novelty of these regions was confirmed by tBlastx analysis of the nucleotide sequence (results not shown). These regions may have been acquired through horizontal gene transfer. These regions span ORFs 2–5, 15–20, 31–32 and 46–47. The first of these regions includes four genes of unknown function (ORFs 2–4) and ORF 5, which encodes a predicted dksA (DnaK suppressor) transcriptional regulator. The protein encoded by ORF 4 showed 85 % identity to a hypothetical protein encoded by a gene in the *P. arcticus* 273-4 genome that lies outside of the predicted Psyar273-4/1 prophage region.

The second region, from ORF 15 to 20, encodes hypothetical proteins of unknown function, includes a tRNA gene and is flanked by a homing endonuclease gene. The protein encoded by ORF 16 had 42 % identity to a protein encoded by *A. baumannii* SDF, while ORF 18 encoded a protein with 51 % identity to a protein encoded by an uncharacterised Mu-like prophage in the *P. arcticus* 273-4 genome. A predicted tRNA gene specific for Lys/Asn at bp 11,082–11,160 was identified in this region. Analysis of the codon usage in Psymv2 revealed a significantly higher number of codons for Lys (3.584 %) compared to the average codon usage of between 0.675 and 2.45 %. The presence of the tRNA Lys/Asn gene in the Psymv2 genome therefore appears to correspond to the high usage of the Lys codon in the Psymv2 genes. Interestingly, Ayala-del-Río et al. (2010) recently showed that cold-adapted proteins encoded by *P. arcticus* 273-4 have an increased Lys content. Amino acid substitutions that increase the structural flexibility of proteins, such as Lys for Arg, have been proposed to be necessary for activity at low temperatures and are thought to be indicators of cold-adaptation (Ayala-del-Río et al. 2010). Flanking this region is ORF 21, which encodes a homing endonuclease containing a conserved active site core motif of the HNHc family (Table 1). Homing endonuclease genes are known as selfish genetic elements and are commonly found in phages and bacteria (reviewed by Marcaida et al. 2010). A homing mechanism is used to copy the sequence, and often surrounding sequences, to the same site at different loci, such as in related phages. In phage T4 the *SegB* homing endonuclease has been shown to be responsible for the transfer of the flanking T4 tRNA region to related phages during co-infection (Brok-Volchanskaya et al. 2008). The HNH endonuclease, and any surrounding co-transferred sequences, may then confer a selective advantage to the phage and/or its lysogen.

The region from ORF 31 to 32 lies within the Psymv2 structural module and may be involved in superinfection exclusion. Both encoded proteins have a TMD and are

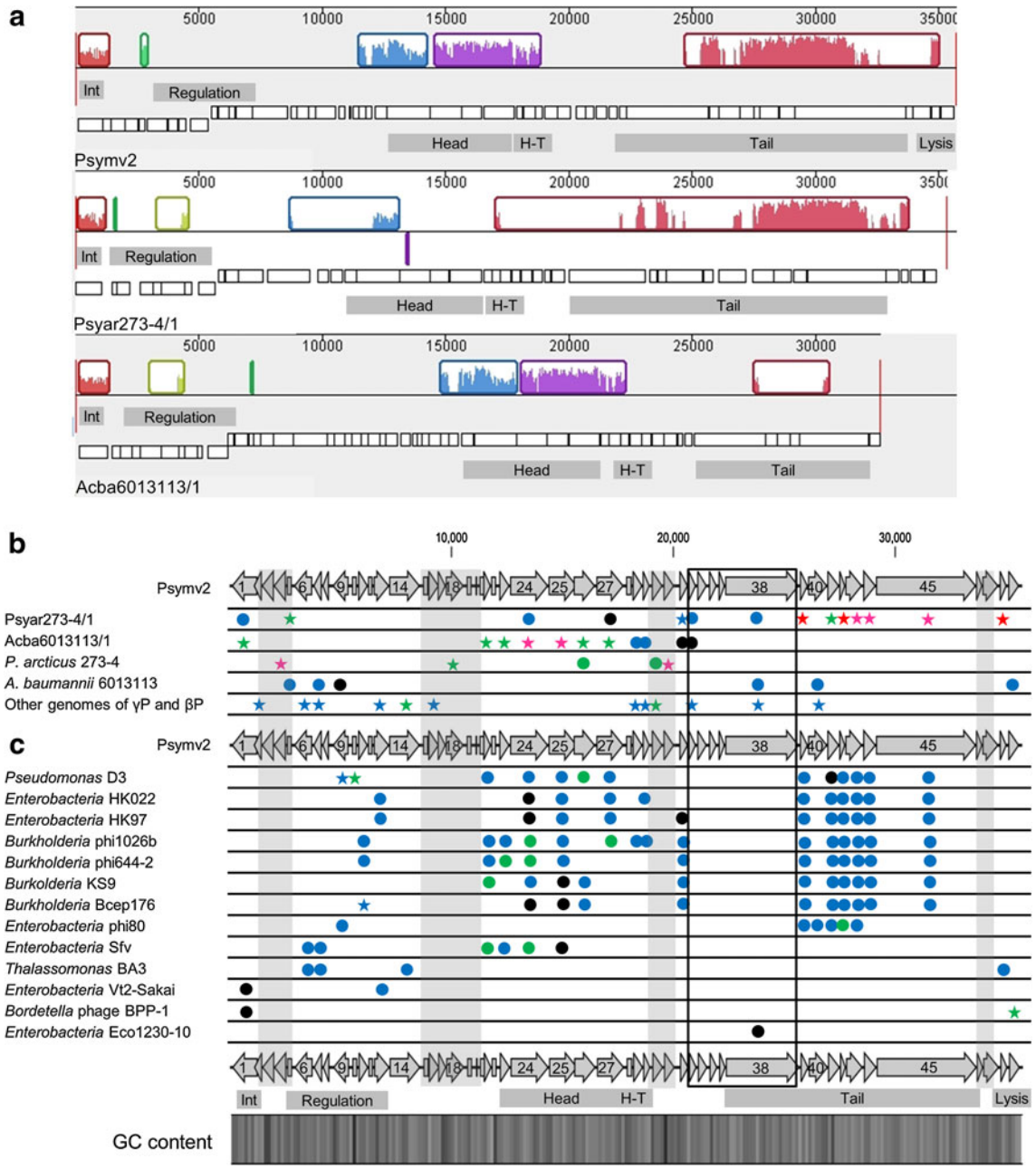

Fig. 3 Comparison of phage Psymv2 nucleotide and protein sequences to related phages. **a** Whole genome alignment of phage Psymv2 and putative prophages within the *P. arcticus* 273-4 and *A. baumanni* 6013113 genomes. Similarly *coloured regions* indicate homology or local collinear blocks (LCB) between nucleotide sequences, with the level of similarity indicated by the height of the *bars* within each LCB. Genome alignments were performed using Mauve. **b** and **c** Blastp hits for Psymv2 proteins against the nr database (**b**) and against proteins of viral origin (virus[Orgn]) (**c**), with an *E*-value of less than 1e-5. *Coloured stars* indicate the top hits for each coding sequence and circles other hits with similarity. The percentage identity between the Psymv2 proteins and the Blastp hits is indicated, with *red* above 90 %, *pink* 70–90 %, *green* 50–70 %, *blue* 30–50 % and *black* less than 30 % identity. The abbreviations γP and βP designate *Gammaproteobacteria* and *Betaproteobacteria* respectively. The *grey* blocks indicate regions with little to no similarity to known phages. The % G + C composition of the Psymv2 genome is indicated by the *bar* at the *bottom* of the figure. A sliding window length of 75 bp was used with % G + C composition scaled from *black* (0 %) to *white* (100 %) (colour figure online)

predicted to be membrane proteins, with the ORF 31 gene product being significantly related to a family of lipoproteins (Table 1). Many phages use membrane targeted proteins that prevent the entry of DNA from superinfecting phage into the cell. The protein encoded by ORF 32 had 84 % identity (57 % query coverage) to a hypothetical protein encoded by *P. arcticus* 273-4. The last region, from ORF 46 to 47, lies between the structural and lysis modules

of Psymv2 and the encoded proteins had no known function or significant homologues.

Psymv2 bacterial integration site

The Psymv2 chromosomal integration site was sequenced by genome walking outwards from both ends of the integrated prophage on the *Psychrobacter* sp. MV2 genome. Analysis of the sequence upstream of the prophage integrase (ORF 1) using Aragorn (Laslett and Canback 2004) identified an *ssrA* gene as the Psymv2 integration site. The *ssrA* gene encodes a small stable tmRNA, with both tRNA- and mRNA-like functions, that is known to be involved in the recovery of stalled ribosomes on degraded mRNA. The tmRNA encodes a short peptide tag which is added to partially synthesised proteins targeting them for degradation (Karzai et al. 2000). Immediately upstream of the *ssrA* gene in the *Psychrobacter* sp. MV2 genome was an ORF encoding a putative phospholipase/carboxylesterase (Fig. 4a) with 90 % identity (*E*-value 5e-116) to a cognate protein encoded by *P. arcticus* 273-4.

The last 27 bp of the *ssrA* gene were found to be directly repeated at the opposite prophage end (Fig. 4a). This 27 bp identical repeat presumably represents the conserved core sequence of the Psymv2 attachment site (*attB*, *attP*, *attL* and *attR*) and functions as the recognition site for Psymv2 integration and excision. The *att* core sequence represents the T-loop of the tRNA region of the *Psychrobacter* sp. MV2 tmRNA (Fig. 4b). The exact repetition of this sequence in the phage genome upstream of the integrase (*attL*) reconstitutes the *ssrA* gene following integration. The ssrA gene was found to be highly conserved in the *P. arcticus* 273-4 genome with 97 % nucleotide identity. Neither Psyar273-4/1 nor Acba6013113/1 were found to be integrated at an *ssrA* gene. The 27 bp Psymv2 att core sequence was, however, found in numerous *Psychrobacter* and *Acinetobacter* genomes (including *A. baumannii* 6013113) with 100 % identity, in many cases flanking an integrase or transposase gene (results not shown). Prophage insertion at *ssrA* genes is estimated to occur for 8 % of predicted prophages (Fouts 2006).

Concluding remarks

In this paper we present the first phage isolated from a member of the genus *Psychrobacter*. Morphologically, the phage can be classified as a *Siphoviridae*. This classification was supported by the fact that many of the phage structural proteins have homology to cognate proteins of several members of this family. The closest relatives in terms of gene order, nucleotide and predicted amino acid sequence similarity were uncharacterised prophages within the genomes of *Psychrobacter arcticus* 273-4 and *Acinetobacter baumannii* 6013113. A more detailed understanding of the phylogeny of Psymv2 was hampered, as is the case with most phages, by the mosaic nature of the genome and the limited number of complete genome sequences of related characterised phages. The availability of additional genomic data from phages infecting bacteria within the family *Moraxellaceae* should no doubt provide greater insight.

A number of interesting and unique ORFs were identified within the Psymv2 genome. These will be the subject of future investigations as they are of interest for their potential involvement in the environmental fitness of the lysogen, *Psychrobacter* sp. MV2. Recent studies have shown that, at least in marine environments, lytic infection appears to be prevalent in highly productive habitats, while lysogeny is more common under unfavourable environmental conditions. Here prophages may support the survival of their host by encoding repressors and

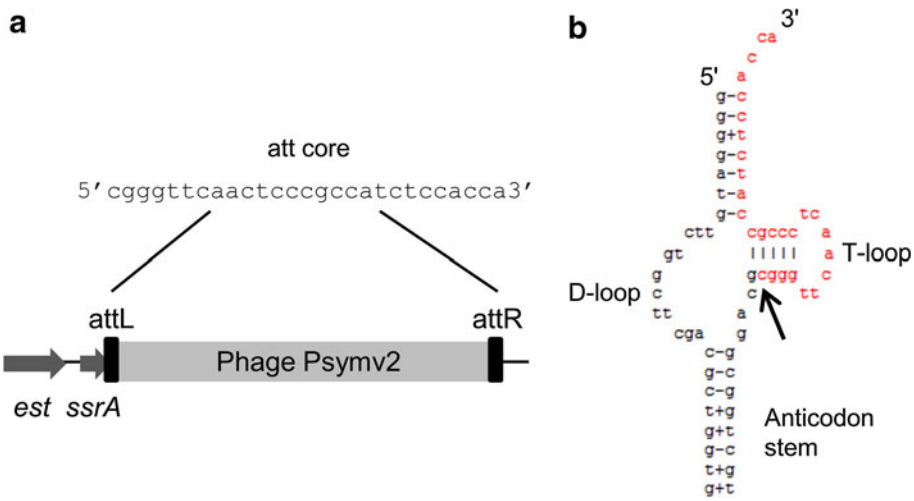

Fig. 4 Integration of phage Psymv2 into the T-loop of the tRNA region of the *Psychrobacter* sp. MV2 *ssrA* gene. **a** The integrated Psymv2 prophage is flanked by an exact 27 bp direct repeat that duplicates the last 27 bp of the 3′ end of the *Psychrobacter* sp. MV2 *ssrA* gene. **b** The tRNA-like domain of the *Psychrobacter* sp. MV2 tmRNA predicted using Aragorn (Laslett and Canback 2004). The Psymv2 phage *att* site is indicated by the *arrow*

transcriptional regulators that inhibit host genes controlling superfluous metabolic processes (Paul 2008).

Viral control of microorganisms is now considered to be an important component of ecological and geochemical processes in most environments. Several bacteriophages infecting deep-sea hydrothermal vent bacteria have been characterised, including the well-studied *Siphoviridae* GVE2; a lytic phage of the thermophile *Geobacillus* sp. E263 (Liu et al. 2006; Liu and Zhang 2008). An analysis of the effects of GVE2 infection on the host cellular proteome revealed phage-induced differential expression of host proteins involved in energy metabolism, replication and transportation of energy and matter (Wei and Zhang 2010). A similar investigation of the response of *Pscyhrobacter* sp. MV2 to infection with Psymv2 would contribute to our understanding of virus-host interactions at low temperature.

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Conflict of interest The authors declare that they have no competing interests.

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