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Characterization and genome analysis of the temperate bacteriophage φSAJS1 from *Streptomyces avermitilis*

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ABSTRACT

Streptomyces is an important antibiotic-producing bacterium; however, antibiotic production is often negatively affected by bacteriophage contamination. In the present study, the temperate phage φSAJS1 was isolated and characterized from an unsuccessful *Streptomyces avermitilis* fermentation culture. The complete genome of phage φSAJS1 was sequenced. Phage φSAJS1 belongs to the *Siphoviridae* family based on its morphology as determined by transmission electron microscopy. Host range analysis indicated that phage φSAJS1 specifically infects various *S. avermitilis* strains. One-step growth curve assays revealed that Ca^{2+} is required for abundant phage proliferation and that phage φSAJS1 is resistant to high temperatures (70 °C) and alkaline solutions (pH 11). The phage φSAJS1 genome is a circular double-stranded (ds) DNA that does not contain terminal repeats and cohensive ends, thereby suggesting a headful DNA packaging mechanism. The whole phage φSAJS1 genome is 56,451 bp in length with a high GC-content (68.3%) and encodes 76 putative open reading frames. Similarity analysis showed that the majority of the candidate proteins share high similarity (50%–72%) to proteins in the *S. griseus subsp.* phages YDN12 and TP1604, indicating either a common origin or more recent DNA recombination events throughout the evolution of these three phage lineages.

1. Introduction

Streptomyces avermitilis is a Gram-positive mycelial bacterium with a high GC content. It is an important industrial fermentation microorganism that is used for avermectin production (Kim and Goodfellow, 2002). Avermectin and its derivatives are widely used in agriculture, animal husbandry, and medicine (Liu et al., 2015; Siddique et al., 2014). *S. avermitilis* and other *Streptomyces* species are negatively affected by phage contamination during fermentation, which causes huge economic losses. Contamination of *Streptomyces* fermentation by phages has been reported since 1980s (Diaz et al., 1991; Kuhn et al., 1987; Zhou et al., 1994). However, little is known about *Streptomyces* phages, and only 20 complete phage genomes have been released in public databases. The reported *Streptomyces* phages are all classified under the *Siphoviridae* family; of these, six strains have been studied in detail. The earliest reported *Streptomyces* phage TG1 was isolated from soil by growth on *S. cattleya* and is capable of infecting *S. avermitilis* MA4990 (Foor et al., 1985). *S. coelicolor* is the host of phages phiC31 and phiBT1 (Smith et al., 1999), and *Venezuela velutipes* and *S. striatum* are the hosts of phages VWB and μ1/6, respectively (Farkasovska et al., 2007; Van et al., 2005). We have previously analyzed the *S. avermitilis* phage phiSASD1 (Wang et al., 2010). Notably, the phage phiC31 has been well studied. Many vectors, such as pSET152, have been developed based on phiC31 and have been widely used for genetic manipulation of *Streptomyces*. However, little is known regarding *Streptomyces* phages, especially those that cause contamination of industrial fermentation, and information severely lacking compared to those of other intestinal, dairy, and *Mycobacterium* phages. Studies on *Streptomyces* phages can help reveal the interactions between phage and host and help elucidate the mechanisms behind host's anti-phage mechanisms, such as the CRISPR-Cas system.

Two phages, namely, phiSASD1 and φSAJS1 of *S. avermitilis*, were

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isolated from failed fermentation cultures of *S. avermitilis* in two factories located in two provinces of China, conducted from a previous study. The phage phiSASD1 has been previously described (Wang et al., 2010). Despite having the same host range, these two phages show many differences in terms of general biological characteristics and genomes. Here, we report the identification, characterization, and genome analysis of phage φSAJS1. The current findings could serve as the basis for the prevention and control of *Streptomyces* phages during industrial production and can facilitate the development of vectors based on the phage genome. In addition, our results can shed light on the phage evolution and phage-host interactions.

2. Materials and methods

2.1. Preparation and purification of phage, one-step growth assay, and electron microscopy

Phage φSAJS1 was isolated from a fermentation factory and stored in the laboratory following the standard agar-layer technique (Vats et al., 1987). Phage lysates were prepared with *S. avermitilis* ATCC 31267 following the standard agar-layer technique (Vats et al., 1987). Purification of the phage particles and one-step growth experiment were performed as previously described (Wang et al., 2010). Phages particles were visualized on a Hitachi H-7650 electron microscope operated at 80 kV with magnifications ranging from 8000× to 20,000×.

2.2. Thermal and pH stability assays

To investigate the thermal stability of phage φSAJS1, 1 mL of phage φSAJS1 (~10^7 PFU/mL) in sodium chloride-magnesium sulfate (SM) buffer (100 mM NaCl, 50 mM Tris, 10 mM $MgSO_4$, and 0.01% gelatin, pH 7.5) was incubated at 30, 40, 50, 60, 70, 80, and 85 ℃ for 2 h, after which the phage suspension was immediately cooled to 4 ℃ for survival estimation by the double-layer agar method. To evaluate the stability of phage φSAJS1 in solutions under various pH conditions, 1 mL of phage φSAJS1 phage suspension (~10^7 PFU/mL) was added to 9 mL of 1% peptone solution with various pH values and subsequently incubated at 30 ℃ for 24 h. Phage viability was determined by the double-layer agar method. All assays were performed in triplicate.

2.3. Lysogenic/lytic assays

To investigate the lysogeny of phage φSAJS1, 10 µL of phage suspension (~10^9 PFU/mL) was added dropwise onto double-layer agar plates inoculated with *S. avermitilis* ATCC 31267. After growth, the cells in the center portion of the plaques were carefully pipetted out and inoculated onto new plates. After two rounds of isolation and purification, seven randomly selected bacterial colonies were used for colony polymerase chain reaction (PCR) using one pair of primers targeting ORF19 (major capsid protein) (forward primer 5′-GCCCATG GCCGACATCTACG-3′ and reverse primer 5′-GGACGGGCCAACGGACT AGG-3′). PCR was performed using chromosomal *S. avermitilis* DNA based on the following protocol: 95 ℃ for 5 min, followed by 25 cycles of 95 ℃ for 1 min, 64 ℃ for 1 min and 72 ℃ for 1 min. PCR products were resolved by electrophoresis on a 0.8% agarose gel. To further confirm the entry of the phage genome into host cells, Southern blotting analysis was performed following a standard procedure (Sambrook et al., 1989).

2.4. Topology analysis of phage genome in the mature phage particles

To analyze the topology of the phage genome, 2 mL of the phage lysate was adjusted to about pH 12.3 with 5 M NaOH, and 15 µL of the sample was collected. Afterwards, the lysate was adjusted to about pH 8.0 with 5 M HCl, and 15 µL of the sample was collected. Then, the two samples with different pH values were resolved by electrophoresis on 0.7% agarose gel.

2.5. Phage DNA extraction, restriction endonuclease digestion and agarose gel electrophoresis, sequencing, and genomic analysis

Phage DNA extraction was performed as described in Sambrook et al. (1989). Briefly, purified phage particles were treated with SDS/proteinase K to remove the proteins, and DNA was extracted by phenol/chloroform extraction.

Restriction endonuclease digestion and agarose gel electrophoresis were performed following standard procedures (Sambrook et al., 1989). Restriction endonucleases were obtained from Takara Bio Inc. (Dalian, China).

The genome of phage φSAJS1 was sequenced by the shotgun method with an average 8.6-fold redundancy at the Beijing Genomics Institute (Beijing, China). Sequence reads were analyzed using the Phred/Phrap/Consed program (Ewing and Green, 1998; Gordon et al., 1998). Genomic analysis of phage φSAJS1 was performed using various biological information prediction software as previously described in Wang et al. (2010).

2.6. Genome sequence accession number

The genome sequence of phage φSAJS1 was deposited in GenBank under the accession number KT989433 (https://www.ncbi.nlm.nih.gov/search/?term=KT989433).

3. Results

3.1. Isolation and morphology of phage φSAJS1

Phage φSAJS1 was isolated from a failed fermentation of *S. avermitilis* in the factory located in Jiangsu province of China. Negatively stained particles showed that the head of phage φSAJS1 has a globular shape with a diameter of 74 nm and a long tail of 227 nm. Phage φSAJS1 is assigned to the *Siphoviridae* family because of its globular head and elongated tail (Fig. 1). Interestingly, a trail is frequently observed in the center of the virus particles. The trail might be a crack due to phage fracture or an appendage in the surface of virus particles (Fig. 1).

3.2. Host range

Host range analysis showed that spores, mycelia, or protoplasts of various of *S. avermitilis* strains, including wild-type *S. avermitilis* ATCC 31267, two mutants *S. avermitilis* 76-09 and *S. avermitilis* 76-05 strongly producing avermectin, and a green spore mutant *S. avermitilis* UA-G, were all sensitive to phage φSAJS1 infection. However, the phage φSAJS1 formed different plaques on the lawns by different strains. As shown in Fig. 2, phage plaques formed by phage φSAJS1 were small and turbid in the tested strains, and some lysogens grew at the center of the plaques and spontaneously released phage particles. The other detected *Streptomyces* species, including *S. coelicolor* M145, *S. lividans* TK54, *S. nigrificans*, *S. violaceorectus*, *S. venezuelae*, *S. rimosus* 10970, and *S. toyocaensis*, were found to be insensitive to phage φSAJS1.

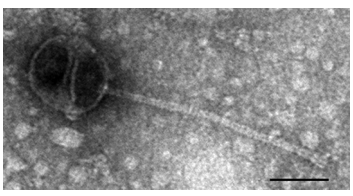

Fig. 1. Electron micrograph. Magnification, 20,000×; scale bar, 50 nm.

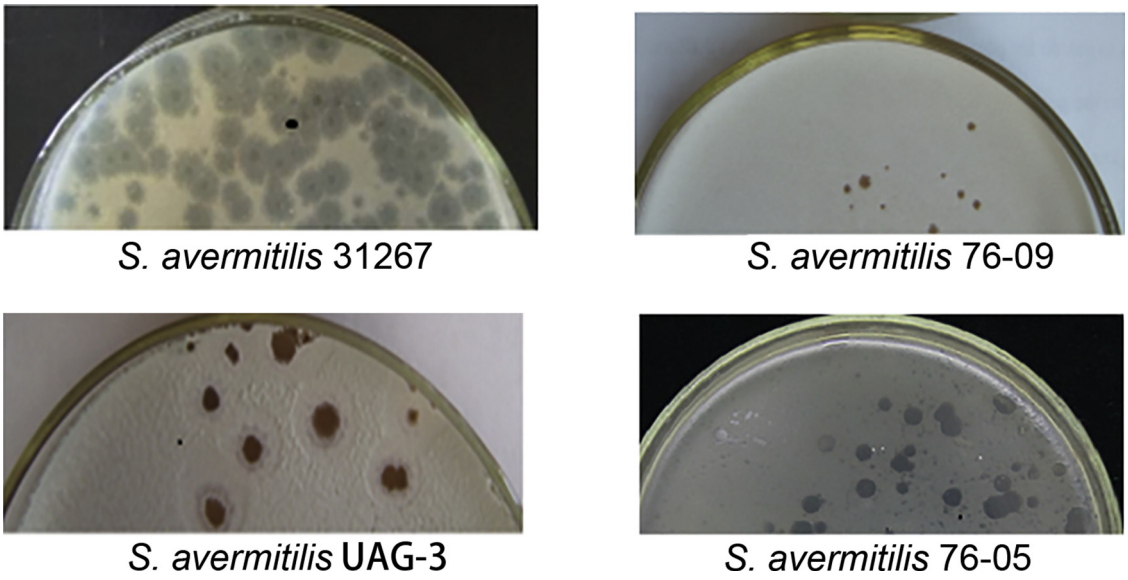

Fig. 2. Plaques formed by phage φSAJS1 on different *S. avermitilis* strains.

3.3. Thermal/pH stability assessment

Stability assessment showed that the activity of phage φSAJS1 was stable between 30 ℃ and 70 ℃. Phages maintained at 80 ℃ for 1 h showed 90% reduction in viability rate relative to that obtained at 30 ℃. Furthermore, phage φSAJS1 lost nearly all activity when incubated at a temperature higher than 85 ℃ (Fig. 3A). Results of the pH stability test showed that the pH tolerance of phage φSAJS1 ranges from 4 to 13 with an optimal pH of 7. In addition, the phage survival rate was higher than 90% between pH 7 and pH 11 after 24 h of incubation. However, phage activity was completely abolished when the pH was maintained below 4 (Fig. 3B).

3.4. One-step growth curve

A previous study showed that specific concentrations of Ca^{2+} and Mg^{2+} are favorable for phage proliferation (Wang et al., 2010). The double-plate method was performed to determine the effects of the two ions on the proliferation of phage φSAJS1. Results showed that the optimal Ca^{2+} concentration for phage φSAJS1 was 10 mM, and increasing the concentration up to 100 mM delayed the appearance of plaques. The low Mg^{2+} concentration (10 mM) exerted little effect on the proliferation of phage φSAJS1, whereas a high Mg^{2+} concentration (50 mM) exerted an inhibitory effect. The effects of the two ions on phage φSAJS1 proliferation were more evident in TSB liquid medium. At 10 mM Ca^{2+}, the one-step growth curve of phage φSAJS1 showed that the latent period is approximately 4 h and the burst size is about 69 PFU/cell (Fig. 4).

3.5. Lysogeny assay of phage φSAJS1

After culture on a double-layer plate coated with an excess of phage φSAJS1, the remaining colonies of *S. avermitilis* ATCC 31267 were isolated. Seven colonies (JS-31267-1 to 7) were selected, and genomic DNA was extracted. One pair of primers was designed based on the ORF19 sequence of phage φSAJS1. The obtained PCR fragment was sequenced, and results confirmed that the ORF19 sequence of phage φSAJS1 was amplified when the genomic DNA samples of seven putative lysogeny hosts were used as PCR templates (Fig. 5A). The above results indicated that the phage genome entered the host cells, which was further confirmed by Southern blotting (Fig. 5B). After Southern hybridization, the genomic DNA of strains JS-31267-1 and JS-31267-2

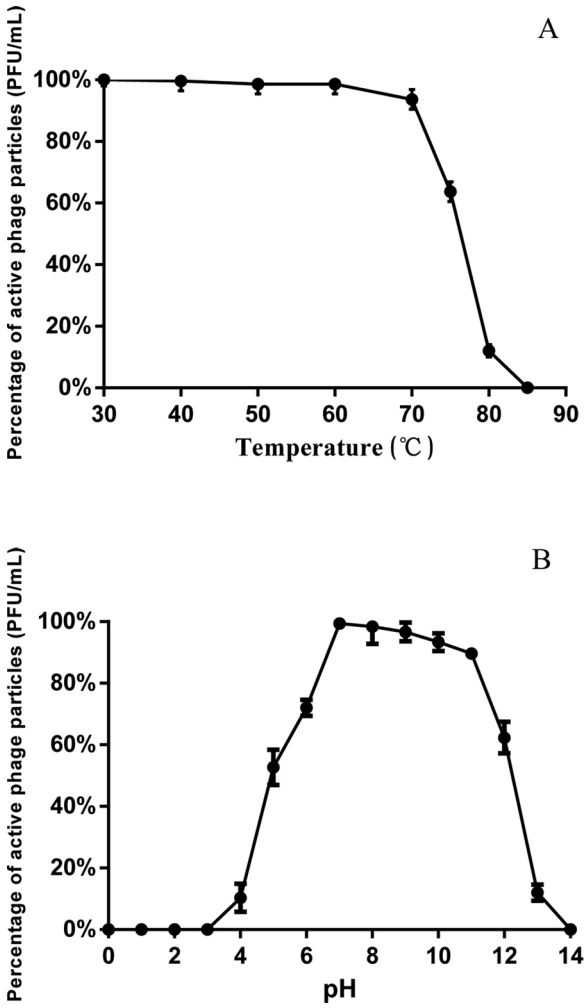

Fig. 3. Stability of phage φSAJS1 under various stress conditions. (A) temperature stability and (B) pH stability. PFU, Plaque Forming Unit. Error bars show standard deviations among triplicate samples.

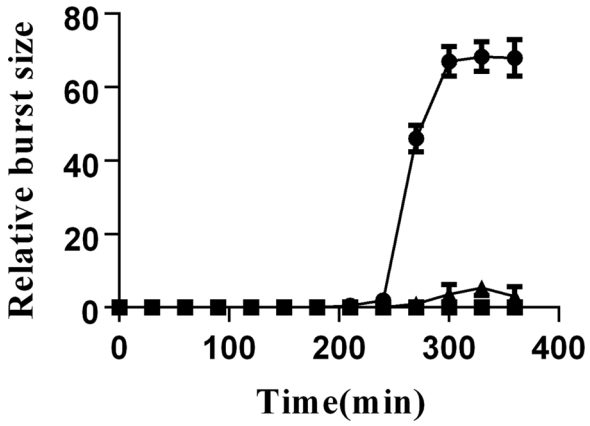

Fig. 4. One-step growth curve of phage φSAJS1 on *S. avermitilis* ATCC 31267 with different cations. ●, 10 mM $Ca(NO_3)_2$; ▲, 10 mM $MgSO_4$; ■, no addition.

produced obvious bands in the corresponding positions as those produced by the genomic DNA of phage φSAJS1; for the wild-type *S. avermitilis* ATCC31267 strain, no bands were detected in the corresponding positions. In addition, results of resistance experiments revealed that the lysogen strains JS-31267-1, JS-31267-2, and JS-31267-3 were resistant to phage φSAJS1 infection, whereas the wild-type strain *S. avermitilis* ATCC31267 was susceptible and formed turbid plaques (Fig. 6).

3.6. Genome of phage φSAJS1

The genome of phage φSAJS1 consists of a double-stranded DNA with a length of 56,451 bp and a G + C content of 68.33%, which was comparable to that of the host genome. When the phage particles were treated with ˜pH 12.3 solution and then adjusted to pH 8.0, the same results, two apparent DNA bands, were identified for the two treatments after agarose gel electrophoresis (Fig. 7A), indicating that the phage genome was resistant to the alkaline solution and is capable of forming covalently closed circular double-stranded DNA molecules in mature phage particles. Results of single and double restriction enzyme digestion of the phage genomic DNA further demonstrated that the genomic DNA of phage φSAJS1 is circular (Fig. 7B and data not shown, respectively). In addition, rapid or slow annealing experiments on the digested genomic DNA after heating at 65 °C for 5 min showed similar results, indicating that the genomic DNA of phage φSAJS1 does not contain a cohensive end (data not shown). No gene encoding tRNA was detected in the genome of phage φSAJS1. The genome structure of phage φSAJS1 is compact, allowing frequent overlaps among ORFs. The longest predicted overlap is a 34-bp overlap between ORF28 and ORF29.

3.7. Identification and analysis of ORFs

The putative ORFs were predicted using various computational software. The phage φSAJS1 genome comprises total of 56,451 bp corresponding to 76 ORFs with sizes ranging from 137 to 3818 bp (Table 1). The temporal and functional distributions of genes are tightly organized and packaged close to each other. All predicted protein-coding genes were screened using BLASTP and Psi-BLAST algorithms against the NCBI non-redundant protein database. Among the 76 coding sequences (CDSs) of phage φSAJS1, 16 (21.1%) have assigned functions. In addition, genes starting with ATG or GTG were observed at the same frequency. Based on overall protein homology, phage φSAJS1 shares the highest similarity with *S. griseus subsp.* phages YDN12 and TP1604 (Fig. 8). Among the 16 ORFs with known functions, 11 ORFs exhibited strong homology with those of the YDN12 and TP1604 phages.

To achieve successful infection, a bacteriophage requires the machinery for gene expression, gene regulation, DNA replication, phage capsid formation, and release of new phage particles from the infected host. The genes in most phages that encode the above basic functions are clustered according to their biological function and are divided into functional modules (Botstein, 1980; Casjens et al., 1992). Inspection of the identified functions encoded in the phage φSAJS1 ORFs revealed that the genome can be divided into “functional modules”, including tail structural components and assembly, head structural components and assembly, DNA packaging, and the DNA replication and the lysis modules (Fig. 8). The following results were obtained:

(1) Tail structural components and assembly. The functional module for tail structural components and assembly is proposed to cover at least ORF23 to ORF26. Results of PSI-BLAST search revealed significant similarity between ORF23 (59% overall identity) and the tape measure protein (TMP) of *Streptomyces* phage Xkcd426. Furthermore, phage φSAJS1 is a long-tailed phage, and all long-tailed phages possess a large gene encoding a TMP, which can be used for precise determination of the tail length (Pell et al., 2009).

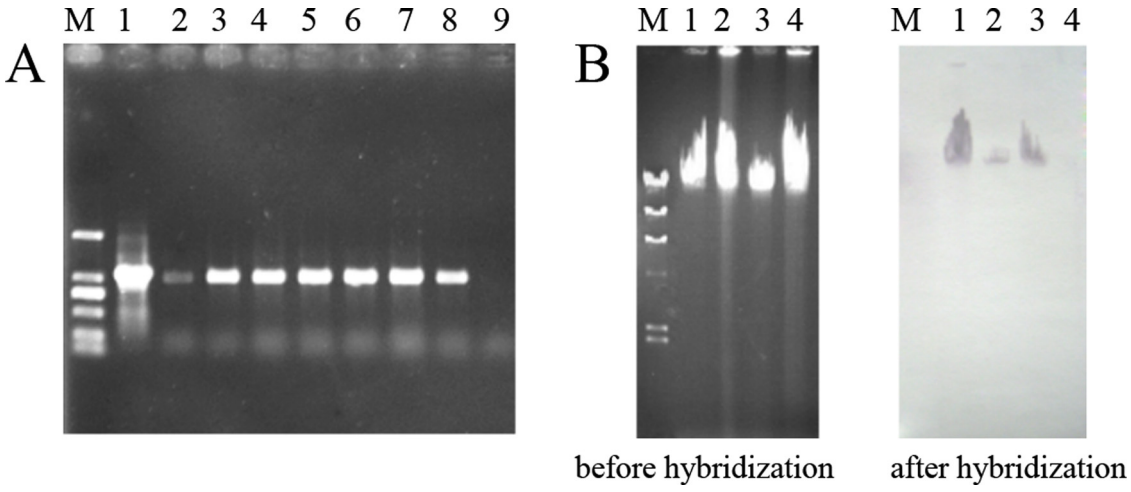

Fig. 5. (A) Agarose gel electrophoresis of PCR products from lysogenic strains. Lane 1, phage φSAJS1; Lane 2, JS-31267-1; Lane 3, JS-31267-2; Lane 4, JS-31267-3; Lane 5, JS-31267-4; Lane 6, JS-31267-5; Lane 7, JS-31267-6; Lane 8, JS-31267-7; Lane 9, *S. avermitilis* ATCC 31267; M, DL2000 ladder marker. Lane 1 is a positive control and Lane 9 is a negative control. (B) Electrophoresis gel of lysogenic strain DNA and Southern hybridization. M. λ *Hind* III ladder marker; Lane 1, φSAJS1; Lane 2, JS-31267-1; Lane 3, JS-31267-2; Lane 4, *S. avermitilis* ATCC 31267.

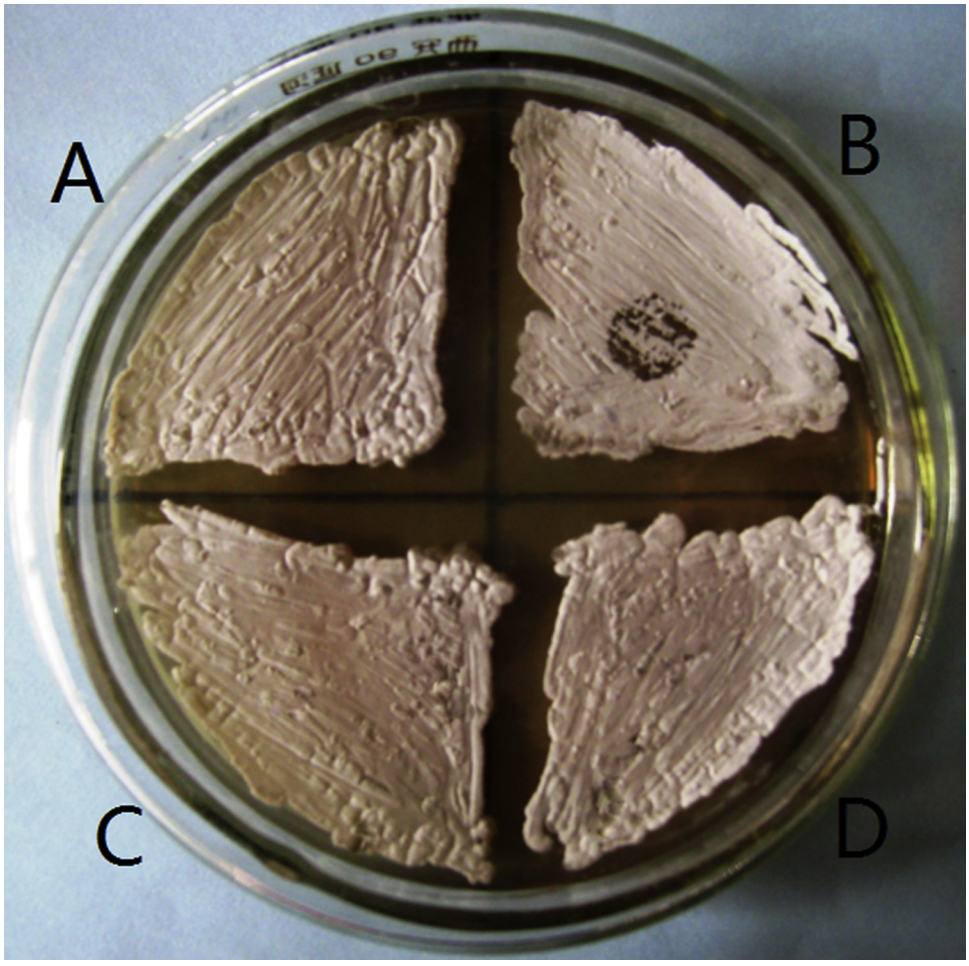

Fig. 6. Resistance of lysogens to lysis of phage φSAJS1. A. JS-31267-1; B. *S. avermitilis* ATCC31267; C. JS-31267-2; D. JS-31267-3.

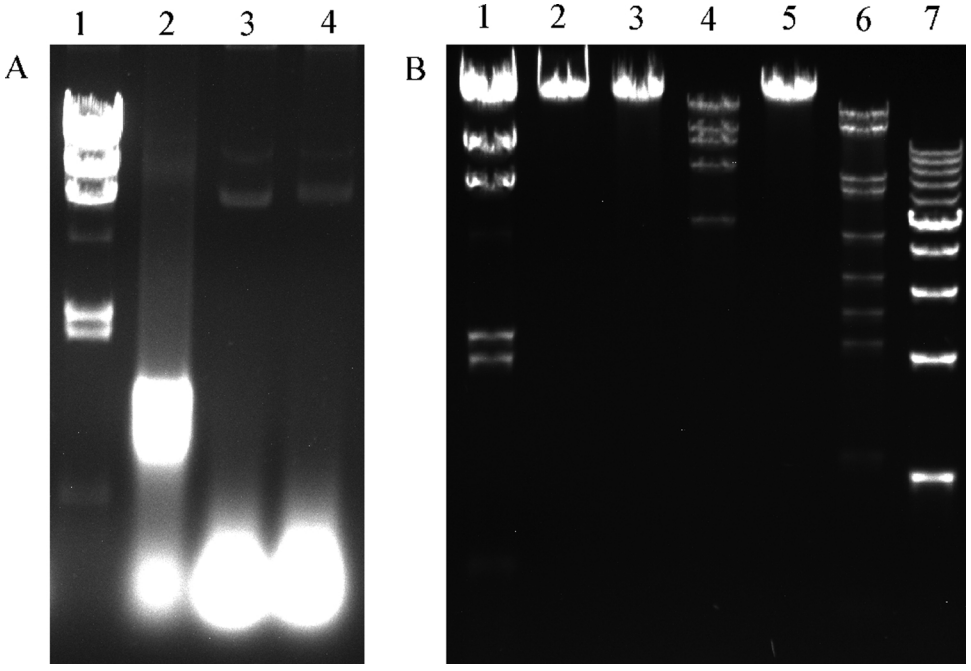

Fig. 7. Agarose gel electrophoresis of phage φSAJS1 particles treated with an alkaline solution (A) and genomic DNA of phage φSAJS1 digested with five types of restriction enzymes (B). (A) Lane 1, phage φSAJS1 lysate; Lane 2, phage particles were treated with a pH of ˜12.3 solution; Lane 3, the pH of phage particles in Lane 2 was adjusted to ˜8.0. (B) Genomic DNA of phage φSAJS1 was completely digested with *Eco*R V, *Bam*H I, *Sac* II, or *Apa*L I. Lane 1, λ Hind III ladder marker; Lane 2, phage φSAJS1 genomic DNA; Lane 3, *Eco*R V; Lane 4, *Bam*H I; Lane 5, *Sac* II; Lane 6, *Apa*L I; Lane 7, 1-kb ladder.

Table 1
Phage φSAJS1 gene annotation.

ORF	Putative product	Start(bp)	Stop (bp)	Evidence or Organism	Homolog (Accession number)	Id (%)	BlastP E-value
ORF01	–	1	486	–	–	–	–
ORF02	–	637	1101	–	–	–	–
ORF03	terminase large subunit	1104	2474	*Streptomyces* phage TP1604	YP_009200118.1	77	0
ORF04	–	2561	2845	–	–	–	–
ORF05	–	2944	3357	–	–	–	–
ORF06	–	3396	3665	–	–	–	–
ORF07	–	3727	4092	–	–	–	–
ORF08	–	4150	4371	–	–	–	–
ORF09	–	4382	4648	–	–	–	–
ORF10		4810	4968	–	–	–	–
ORF11	phage protein	5138	5812	*Streptomyces* phage YDN12	YP_009215320.1	58	$5e^{-86}$
ORF12	head-to-tail connector protein	5893	6678	*Streptomyces* phage Xkcd426	AMD42764.1	43	$5e^{-62}$
ORF13	–	6678	7250	–	–	–	–
ORF14	head-to-tail connector protein	7297	7674	*Streptomyces* phage Xkcd426	AMD42766.1	77	$5e^{-54}$
ORF15	–	8034	8390	–	–	–	–
ORF16	portal protein	8394	10154	*Streptomyces* phage Xkcd426	AMD42752.1	70	0
ORF17	MuF-like capsid morphogenesis protein	10151	13921	*Streptomyces* phage BabyGotBac	APZ82179.1	61	0
ORF18	–	13924	14103	–	–	–	–
ORF19	major capsid protein	14219	15955	*Streptomyces* phage maih	ALY07263.1	58	0
ORF20	–	16091	16909	–	–	–	–
ORF21	–	16900	17331	–	–	–	–
ORF22	–	17328	17714	–	–	–	–
ORF23	tape measure protein	17789	21607	*Streptomyces* phage Xkcd426	AMD42749.1	59	$4e^{-157}$
ORF24	–	21660	22316	–	–	–	–
ORF25	–	22771	23190	–	–	–	–
ORF26	minor tail protein	23184	24551	*Streptomyces* phage Abt2graduatex2	ATN93757.1	56	$2e^{-69}$
ORF27	–	24555	25604	–	–	–	–
ORF28	–	25597	25923	–	–	–	–
ORF29	–	25889	26074	–	–	–	–
ORF30	–	26076	26723	–	–	–	–
ORF31	PE-PGRS family protein	26869	27585	*Streptomyces sp.* SPB074	EDY44007.1	41	$3e^{-04}$
ORF32	–	27601	28437	–	–	–	–
ORF33	–	28480	30210	–	–	–	–
ORF34	–	30247	30423	–	–	–	–
ORF35	–	30427	31368	–	–	–	–
ORF36	–	31398	31658	–	–	–	–
ORF37	–	31722	31946	–	–	–	–
ORF38	–	32038	32349	–	–	–	–
ORF39	–	32438	32881	–	–	–	–
ORF40	–	32884	33321	–	–	–	–
ORF41	–	33318	33656	–	–	–	–
ORF42	–	33653	34006	–	–	–	–
ORF43	–	34103	34336	–	–	–	–
ORF44	–	34349	34963	–	–	–	–
ORF45	–	35021	35251	–	–	–	–
ORF46	WhiB family transcription factor	35256	35726	*Streptomyces* phage Abt2graduatex2	ATN93713.1	48	$4e^{-19}$
ORF47	–	35785	36123	–	–	–	–
ORF48	–	36249	37193	–	–	–	–
ORF49	–	37190	37384	–	–	–	–
ORF50	MazG nucleotide pyrophosphohydr-olase domain	37410	37745	*Mycobacterium abscessus subsp.* abscessus	SIB58123.1	53	$1e^{-29}$
ORF51	–	38005	38181	–	–	–	–
ORF52	–	38168	38521	–	–	–	–
ORF53	phage protein	38654	39808	*Streptomyces* phage TP1604	YP_009200159.1	71	$7e^{-179}$
ORF54	–	39890	40081	–	–	–	–
ORF55	–	40098	40685	–	–	–	–
ORF56	DNA polymerase I	40769	42748	*Streptomyces* phage YDN12	YP_009215352.1	72	0
ORF57	DNA primase	42850	45075	*Streptomyces* phage TP1604	YP_009200164.1	67	0
ORF58	–	45094	45231	–	–	–	–
ORF59	–	45706	46050	–	–	–	–
ORF60	–	46047	46358	–	–	–	–
ORF61	DNA helicase	46472	48373	*Streptomyces* phage TP1604	YP_009200169.1	69	0
ORF62	–	48464	48892	–	–	–	–
ORF63	–	48997	49539	–	–	–	–
ORF64	–	49597	50043	–	–	–	–
ORF65	RuvC_resolvase	50040	50663	*Streptomyces* phage YDN12	YP_009215361.1	50	$3e^{-47}$
ORF66	–	50752	51006	–	–	–	–
ORF67	–	51003	51293	–	–	–	–
ORF68	–	51403	52386	–	–	–	–
ORF69	–	52476	53243	–	–	–	–
ORF70	–	53243	53458	–	–	–	–
ORF71	–	53571	54107	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

ORF	Putative product	Start(bp)	Stop (bp)	Evidence or Organism	Homolog (Accession number)	Id (%)	BlastP E-value
ORF72	–	54172	54384	–	–	–	–
ORF73	–	54580	54939	–	–	–	–
ORF74	–	55031	55534	–	–	–	–
ORF75	–	55564	55983	–	–	–	–
ORF76	–	56051	56266	–	–	–	

Genes are listed by number, along with their predicted function, followed by the nature of the evidence that supports the functional classification. - represents unknown.

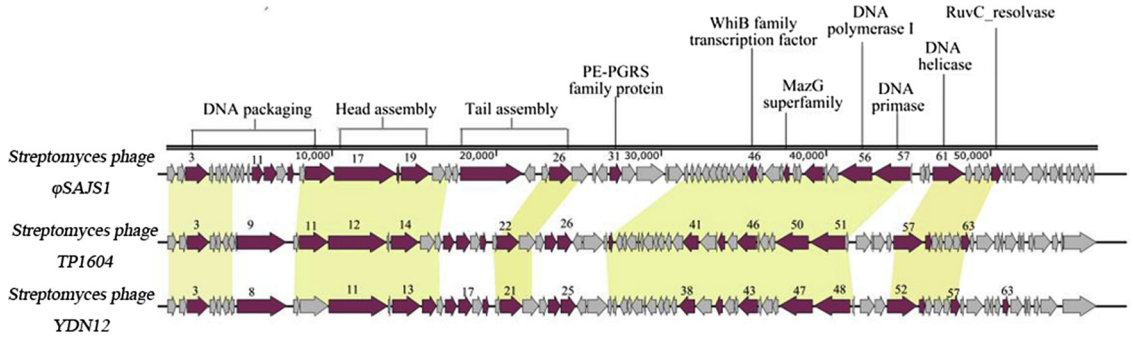

Fig. 8. Genome comparison of *S. avermitilis* phage φSAJS1 and the *S. griseus subsp.* phages YDN12 and TP1604. The ORFs with predicted functions are shown in red color, while those with unknown function are colored in grey.

Thus, based on the observed similarities, ORF23 is likely to function as a tail length tape-measure protein in phage φSAJS1; and similar to most phage TMPs, ORF23 contains up to 72.96% α-helix. Results of InterPro analysis showed that ORF23 contains six transmembrane regions (TMM). Moreover, ORF26 was found to exhibit significant similarity (53% overall identity) to the minor tail protein of *Streptomyces* phage Abt2graduatex2, *S. griseus subsp.* phages YDN12 and TP1604. No proteins showed significant similarity to ORF24 and ORF25.

(2) Head structural components and assembly. The module for the head structural components and assembly of phage φSAJS1 involves ORF17 to ORF19 based on their similarity to the head proteins found in other phages. A PSI-BLAST analysis predicted that ORF17 encodes the MuF-like capsid morphogenesis protein and exhibits 61% overall identity to that of *Streptomyces* phage BabyGotBac and the *S. griseus subsp.* phages YDN12 and TP1604. The predicted product of ORF19 is a major capsid protein because it exhibits 58%–61% similarity to the capsid proteins of various *Streptomyces* phages. However, no proteins showed significant similarity to ORF18.

(3) DNA packaging. Packaging of the bacteriophage double-stranded DNA into the viral capsid relies on a molecular "motor" comprising several proteins, namely, the portal protein located at one vertex of the capsid, and the terminase (Truong et al., 2016). The predicted protein products of ORF03 and ORF16 in phage φSAJS1 were identified as the terminase large subunit and portal proteins, respectively. During DNA packaging, the portal protein associates with the terminase protein (Black, 1989; Rao and Feiss, 2008). The terminase is a component of the molecular motor that translocates genomic DNA into an empty capsid during DNA packaging (Black, 1989).

(4) DNA replication. We propose that the replication module of phage φSAJS1 comprises ORF56 to ORF65 of the genome, considering that several functions related to DNA replication, including endonuclease, helicase, primase, and DNA polymerase, are located within this region. These genes were found to exhibit significant similarity (50%–72% overall identity) to those found in other *Streptomyces* phages, such as *S. griseus subsp.* phage YDN12 and TP1604 based on PSI-BLAST search.

ORF56 of phage φSAJS1 encodes a predicted DNA polymerase I, which has a conserved DNA_pol_A region. The gene product shares strong similarities with ORF55 of the *actinomycete* phage phiAsp2 and ORF11 of phiBT1 and phiC31, as well as many DNA polymerases found in the *mycobacteriophage* phage (Jarling et al., 2004; Smith et al., 1999).

The ORF61 gene in phage φSAJS1 genome encodes a the putative helicase. ORF61 belongs to the DEXDc superfamily and contains four ATP binding sites and a putative Mg^{2+} binding site. During DNA replication, double-stranded DNA is unwound by a helicase in the presence of ATP, and the primase uses a short RNA fragment as a primer to catalyze DNA synthesis by DNA polymerase. Some phages, such as T4 and T5, use separate helicase and primase proteins to form primers, whereas some phages use a bifunctional protein that acts both as a helicase and primase (Ilyina et al., 1992). Phage φSAJS1 is likely belong to the former type of phages because it contains a putative primase, which is encoded by ORF57 and contains a conserved primase_Cterm superfamily region.

The primary function of the DNA endonuclease is the cleavage of the Holliday junction. In phages, endonucleases are likely to play roles in repairing broken DNA by homologous recombination. The five types of DNA endonucleases include T7endoI, T4endoVII, RusA, Rap, and RuvC-like. The putative DNA endonuclease of phage φSAJS1 is encoded by ORF65 and contains a conserved RuvC_resolvase region. Therefore, the DNA endonuclease of phage φSAJS1 belongs to the RuvC-like class. In addition, it exhibits high similarity with ORF46 of phiAsp2 (Jarling et al., 2004), and DNA endonucleases found in other *Mycobacterium* phages, such as ORF8 of Phlyer, ORF8 of Pipefish, and ORF7 of Phaedrus (Hatfull et al., 2010). However, the functions of ORF58-ORF60 and ORF62-ORF64 remain to be investigated.

The lysis module. The final stage of the phage lytic cycle is degradation of the bacterial cell wall and release of progeny phages. The lysis of the cell wall is typically induced by two phage-encoded proteins, namely, holin and endolysin (Hanlon, 2007; Kutter and Sulakvelidze, 2005). Phage φSAJS1 encodes one lysis protein, ORF35; however, no holin protein could be predicted. The product of ORF35 was predicted to be a phage lysis protein because it showed 54% and

52% similarity to lysis proteins encoded in *Streptomyces* phage Xkcd426 and *Streptomyces* phage TP1604, respectively. BLAST analysis identified a putative N-acetylmuramoyl-L-alanine amidase domain (pfam01510) at 40aa to 182aa of ORF35 and a putative peptidoglycan binding domain (pfam01471) at 246aa to 296aa.

4. Discussion

In the present study, we investigated the biological and genomic characteristics of phage φSAJS1. The phage φSAJS1 genome has a size of 56.4 kb, which lies within the common size range of temperate phages genomes. The phage φSAJS1 genome has a G + C content of 68.33%, which is close to that of the host genome and indicates its long-term evolutionary relationship with the host. The above findings are consistent with the previously reported temperate phages and their hosts' GC content (Nakayama et al., 1999). Phage φSAJS1 has a narrow host range, and many of the tested *Streptomyces* strains cannot be infected by phage φSAJS1, except for *S. avermitilis*, indicating that phage φSAJS1 is host-specific. Previous studies suggested that the number of tRNAs is positively correlated with host range because of the compensation for different codon usage patterns in host bacteria (Hyman and Abedon, 2010). No tRNAs were identified in the phage φSAJS1 genome, which could explain its narrow host range. In addition, results of stability assessment showed that phage φSAJS1 is highly resilient to temperature and alkali conditions. As shown in Fig. 3B, phage φSAJS1 activity was almost completely lost at pH values below 4, indicating that it is sensitive to acidic conditions. Therefore, the addition of acid can be performed in applications where industrial fermentation is contaminated by phage φSAJS1 to control the reproduction of phage φSAJS1 and ensure greater fermentation yield.

Morphological and molecular analyses indicated that phage φSAJS1 is a temperate phage. Morphologically, as shown in Fig. 2, phage plaques formed by phage φSAJS1 in the tested strains were small and turbid; on the other hand, some lysogens grew at the center of the plaques and spontaneously released phage particles. In the resistance experiments the lysogen strains JS-31267-1, JS-31267-2, and JS-31267-3 were found to be resistant to phage φSAJS1 infection, whereas the wild-type strain *S. avermitilis* ATCC31267 was susceptible, leading to the formation of turbid plaques (Fig. 6). The results of lysogenic/lytic assay indicated that bacterial isolates from the central region of plaques showed positive PCR amplification using primers specific to phage φSAJS1 ORF19 and Southern blotting of lysogens (Fig. 5). Taken together, the above results indicated that phage φSAJS1 is a temperate phage. However, the phage φSAJS1 is likely to integrate into the genomes of the host cells or exist as covalently closed circular DNA, similar to the P1 phage in *E. coli* (Huang and Masters, 2014). Future studies should investigate the genome state of phage φSAJS1 in the lysogenic strains.

Most double-stranded DNAs (dsDNA) phages employ at least two proteins, namely, endolysin and a small hydrophobic protein called holin, to coordinate a well-timed and rapid host cell lysis (Young et al., 2000). The product of ORF35 was predicted to be a lysis protein. However, no ORFs in phage φSAJS1 exhibited significant homology to holin proteins, indicating the limited number of holin protein sequences deposited in database. In general, some genes that are important for virion assembly and replication and host cell lysis are clustered in phage genomes. Phage φSAJS1 showed a lower number of gene clusters that exhibit significant similarity to other phage proteins with known functions, which could be attributed to the scarcity of genomic information on *Streptomyces* phages. Therefore, additional analyses on *Streptomyces* phage genome sequences and gene function can significantly improve our understanding phage population evolution.

Authors' statement

NL, SW, and YS conceived and designed the experiments. NL, CK, and SW performed the experiments. NL, SW, YS, and YQ analyzed the data. NL wrote the paper. NL, SW, ZC, YW, QW, and YS revised the paper. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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