수의학석사학위논문

Characterization of a T7-like lytic bacteriophage (ϕSG-JL2) of *Salmonella enterica* serovar Gallinarum biovar Gallinarum

Salmonella Gallinarum에 특이적인 T7-like lytic bacteriophage(ΦSG-JL2)의 특성 분석

2013년 2월

서울대학교 대학원

수의학과 수의미생물학 전공

정 지 혜

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February, 2012

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A Dissertation

Submitted to the Graduate School in Partial Fulfillment of the Requirements for Degree of master

To the Faculty of

Department of Veterinary Medicine

The Graduate School

Seoul National University

February, 2012

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수의학 석사학위 논문

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이 논문을 수의학 석사학위논문으로 제출함

2012 년 10 월

서울대학교 대학원

수의학과 수의미생물학 전공

정 지 혜

정지혜의 석사학위논문을 인준함

2012년 12월

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

Abstract

Characterization of a T7-like lytic bacteriophage (ϕSG-JL2) of *Salmonella enterica* serovar Gallinarum biovar Gallinarum

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ϕSG-JL2 is a newly discovered lytic bacteriophage infecting *Salmonella enterica* serovar Gallinarum biovar Gallinarum (*S.* Gallinarum), but is non-lytic to a rough vaccine strain of *S.* Gallinarum(SG-9R), *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella enterica* serovar Gallinarum biovar Pullorum (*S.*

Pullorum). The ϕSG-JL2 genome is 38,815 bp in length (GC content of 50.9% and 230 bp length direct terminal repeats) and 55 putative genes may be transcribed from the same strand. Functions were assigned to 30 genes based on the high amino acid similarity with known proteins. Most of the expected proteins except tail fiber (31.9%) and overall organization of genomes were similar to yersiniophage ϕYeO3-12. ϕSG-JL2 could be classified as a new T7-like virus and represents the first *S.* Gallinarum phage genome to be sequenced. On the basis of intraspecific ratios of nonsynonymous to synonymous nucleotide changes (Pi[a]/Pi[s]), gene 2 encoding host RNA polymerase inhibitor displayed Darwinian positive selection. Pre-treatment of chickens with ϕSG-JL2 before intratracheal challenge with wild-type *S.* Gallinarum protected most birds from fowl typhoid. Therefore, ϕSG-JL2 may be useful for differentiation of *S.* Gallinarum from other *Salmonella* serotypes, prophylactic application to fowl typhoid control, and understanding of vertical evolution of T7-like viruses.

Key words: T7-like virus, *S.* Gallinarum, genome analysis, host adaptation, prophylactic application

Student Number: 2005-22145

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1. Introduction

T7-like viruses have short and non-contractile tails, and are members of the family *Podoviridae*. To date, eight strains have been assigned as *Enterobacteria phage* T7 and three strains (T3, T7, and ϕYeO3-12) have been characterized genomically (http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm) (19, 50, 51). Genetic recombination between T7-like viruses infecting different bacterial genera or different species has been demonstrated, and T3 may have evolved from an ancient phage generated by recombination between yersiniophages ϕA1122 and ϕYeO3-12 (20, 51). Horizontal genetic transfer results in genomic mosaicism of phages, which hinders their hierarchical classification (22, 37). However, common genetic components and lay-outs observed among T7-like viruses may support the idea that they crossed "Darwinian Threshold" and have been undergoing vertical evolution (26, 79). Therefore, they may be useful in understanding genetic variations of closely related T7-like phages during host adaptations. However, current genomic data is not sufficient to permit such detailed analysis. Additional genome sequences of closely related T7-like viruses are required to gain insight into their vertical evolution.

Fowl typhoid is an acute septicemic disease occurring in adult chickens. The disease is characterized by anemia, leukocytosis, and hemorrhage, and is an economically disastrous disease in the poultry

industry (53). The causative agent, *S.* Gallinarum, is classified into serogroup D and is both non-motile and host-adapted (3, 53). Fowl typhoid has been reported to spread via feces-to-oral route but recently it was reproduced by intratracheal challenge of *S.* Gallinarum (4). Differentiation of *S.* Gallinarum from frequent avian serogroup D *Salmonella* such as *S.* Pullorum and *S.* Enteritidis has been partially successful (33, 34), and differentiation of field strains of *S.* Gallinarum from the rough vaccine strain SG-9R has become important because of nation-wide vaccination in some countries. The appearance of multi-drug resistant *S.* Gallinarum strains in the field has prompted increasing concerns about phage therapy, similar to other bacterial diseases (5, 30, 35, 64, 68), but candidate phages that are lytic to broad ranges of *S.* Gallinarum strains have never been reported.

In this study, I report the basic biological properties and complete genomic sequence of a new *Salmonella* T7-like virus, ϕSG-JL2. It is lytic to *S.* Gallinarum and has a double-stranded DNA of 38,815 bp with 55 putative genes. Comparative genomic analyses demonstrate the close relationships of ϕSG-JL2 with ϕYeO3-12 from *Yersinia enterocolitica* O3 and T3 from *Escherichia coli*, and provide molecular clues to understand host adaptations of relative phages. The obligate specificity and broad lytic activity of ϕSG-JL2 may be useful for differentiation of *S.* Gallinarum from *Salmonella* Enteritidis and *S.* Pullorum, and the prophylactic efficacy of ϕSG-JL2 against fowl typhoid was tested with a respiratory model of fowl typhoid.

2. Materials and methods

2.1. Bacteria, phage, and media

S. Pullorum (4 strains) and some *S.* Gallinarum strains used in the present study were identified and reported previously (33, 52). Other *S.* Gallinarum strains were isolated from commercial chickens consigned to diagnosis during 2000 - 2005, and were identified as previously described (52). The SG-9R rough vaccine strain was cultured from commercial live vaccine product (Intervet, Boxmeer, The Netherlands) and reference strains of *Salmonella* Typhimurium (KCTC 12400) and *E. coli* (ATCC 43896) were purchased from the Korea Culture Collection of Microorganisms (Seoul, Korea). *S.* Enteritidis strains (20 strains) were isolated from poultry farms in Korea and identified as previously described (33, 34, 52). All *Salmonella* strains were cultured with MacConkey agar and Tryptic Soy broth (TSB; Difco, Detroit, MI). A lytic *S.* Gallinarum-specific bacteriophage isolated from a sample of final processed sewage water collected in Seoul as described below was designated ϕSG-JL2. Tryptic Soy agar (TSA; Difco) and TSB were used for plaque test and phage propagation as described below.

2.2. Phage isolation, cloning, and propagation

A portion of the final outflow from a sewage processing plant in Seoul was collected and centrifuged at 15,000 *g* for 30 min to

precipitate debris. The supernatant was filtered through a membrane filter with a 0.45 μm pore size. A 26 ml portion of the filtered sewage water was transferred to a 50 ml conical tube. Three milliliters of 10 x TSB and 10^7 colony forming units (CFU)/ml of *S.* Gallinarum 002 (SG002) strain were added, mixed, and incubated at 37℃ for 5 h. The incubated culture was centrifuged (15,000 *g*, 30 min) and the supernatant was diluted 10 fold from 10^{-1} to 10^{-8}. Five hundred microliter of each dilution was mixed with 500 μl of *S.* Gallinarum (10^9 CFU/ml) and plated on a 90 mm diameter TSA plate. A typically large and well-isolated plaque was retrieved with a sterilized yellow tip and suspended in TSB following preparation of 10^{-1} - 10^{-5} dilutions. This process was repeated five times for cloning. The isolated phage was propagated in TSB with the host and filtered through a 0.2 μm pore size membrane filter after centrifugation as detailed above. The plaque forming units (PFU) of the filtered phage was determined as described above. Phage preparations were stored at -70℃ until used.

2.3. Host range determination

A 5 μl volume of each serial dilution (10^{-5} - 10^{-9}) of cloned and filtered phage (10^{10} PFU/ml) was dispensed on lawns of *S.* Gallinarum field strains (106 strains including SG002 and SG101) and SG-9R, *S.* Pullorum(4 strains), *Salmonella* Enteritidis (20 strains), *Salmonella* Typhimurium (KCTC 12400), and *E. coli* (ATCC 43896). PFU was determined after overnight incubation at 37℃.

2.4. Electron microscopy

Purified phages were applied to carbon-shadowed Parlodion-coated grids and stained with 1% uranyl acetate. Electron micrographs of the phage were taken with a Zeiss EM902 transmission electron microscope operating at 80 kV.

2.5. Heat and pH susceptibility test

The heat susceptibility of ϕSG-JL2 was measured at 55℃ for 30 and 60 min together with the host strain, SG002. The pH susceptibility of ϕSG-JL2 was tested at pH 3.0, pH 4.0 and pH 6.0, by mixing equal volume of ϕSG-JL2 with acidic PBS solution (pH 2.0, pH 3.0 and pH 5.0 adjusted with 1M HCl) for 10, 30 and 60 min.

2.6. One-step growth curve

At mid-logarithmic growth phase (determined in preliminary experiments to be at an optical density of 0.5 at 600 nm) SG101 was harvested by centrifugation (15,000 *g*, 15 min) and resuspended in 0.5 volume of the original culture (10^8 CFU/ml). The phage was added at a multiplicity of infection(MOI) of 0.001 and was allowed to adsorb for 5 min. The adsorbed phage and bacteria were centrifuged (15,000 *g*, 15 min) and resuspended in 10 ml of TSB. During the incubation of the resuspension at 37℃ samples were taken at 5 min intervals for 25 min. The samples were immediately diluted and plated for phage titration.

2.7. DNA extraction, cloning, PCR, and sequencing

TSB containing the phage was centrifuged at 15,000 *g* for 30 min and filtered through a 0.22 μm pore size membrane filter. Proteinase K (100 μg/ml) was added and incubated at 65°C for 1 h. Then, an equal volume of phenol/chloroform/isoamyl alcohol (PCI) was mixed with the broth and centrifuged as above. The aqueous phase was collected and the same volume of isopropanol was added. Precipitated phage DNA was collected at 15,000 *g* for 30 min. After washing the DNA by resuspension in 70% ethanol and centrifugation under the same conditions, the phage DNA was resuspended in sterilized deionized distilled water. For the shotgun cloning to obtain partial nucleotide sequences of ϕSG-JL2, the phage genomic DNA and pBluescript II SK (+) was digested with *Hpa*II and *Cla*I, respectively, ligated with T4 DNA ligase, and used to transform competent *E. coli* (Invitrogen, Carlsbad, CA). Inserted DNA was directly amplified by colony-polymerase chain reaction (PCR) with M13 forward and reverse primers as previously described (32). The nucleotide sequences of amplicons were determined using an automatic DNA sequencer with a Dye Terminator kit (Perkin Elmer, Foster City, CA).

The whole genomic nucleotide sequence was determined by aligning the genomic nucleotide sequences of ϕYeO3-12 (AJ251805) and ϕT3 (AJ318471), and designing primer sets from the conserved regions. According to the amplicon nucleotide sequences, additional primer sets were designed to amplify and determine the nucleotide sequences.

Terminal repeat sequences were determined by sequencing of an amplicon that contained right and left terminal repeats (RTL and LTR), and which might originate from the genomic concatemers of ϕSG-JL2 (24). For PCR amplification, 20 μl containing 1 mM $MgCl_2$, 1 mM dNTPs, 10 μM of each forward and reverse primers, and 1 unit of *Taq* polymerase (iNtRON Biotechnology, Sungnam, Korea) were mixed together and PCR was conducted on the mixture at 94℃ for 3 min; 35 cycles of 94℃ for 20 s, 52℃ for 20 s, 72℃ for 90 s; and 72℃ for 7 min. The amplicons were purified with a PCR purification kit (iNtRON Biotechnology) according to the manufacturer's protocol and the nucleotide sequences were determined as described above.

2.8. Sequence analysis

The nucleotide sequences were compared with other genes in GenBank by the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). The open reading frames (ORFs) were identified with the ORF Finder at the National Center for Bioinformatics site (http://www.ncbi.nlm.nih.gov/gorf.html) and GenMark.hmm prokaryotic (Ver.2.5a; http://opal.biology.gatech.edu/GeneMark/). Confirmation was provided by the presence of an appropriately located potential Shine-Dalgarno (SD) sequence up stream of the start codon and comparison of corresponding ORF with those of ϕYeO3-12 and ϕT3.

The molecular weight and isoelectric point were calculated (6) with the Compute pI/Mw program (http://www.expasy.ch/tools/pi_tool.

html). The analogous promoters of host and phage RNAP, Rho-independent terminators, and RNase III recognition sites were manually compared with those of ΦYeO3-12 and ΦT3, and the secondary structures and free energies were calculated with RNA fold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi). The genomic nucleotide sequence of ΦSG-JL2 was compared with those of ΦYeO3-12, ΦT3 and other *Salmonella* phages [SP6 (NC_004831), P22 (NC_002371), ES18 (NC_006949), Gifsy-1 (NC_010392), ST64B (NC_004313), ST64T (NC_004348), Gifsy-2 (NC_010393), Fels-1 (NC_010391), Fels-2 (NC_010463)] with Blast 2 sequences tool (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi), and the synteny plots were generated by the Nucmer program in the Mummer software package (17). The nucleotide and deduced amino acid sequences of phages and hosts genes were aligned by the Clustal method in the MEGA program (32), and the Pi[a]/Pi[s] and Ka/Ks ratios were measured by DnaSP program (Ver. 4.20) (60).

2.9. Prophylactic efficacy of ΦSG-JL2 against fowl typhoid in chickens

To test the prophylactic efficacy of ΦSG-JL2 10^6 CFU/ml of SG101 was treated with 0.1, 1 and 10 MOI of ΦSG-JL2 in tryptic soy broth at room temperature for 4 h. and eighty 13-day-old commercial male brown layer chicks were assigned to one control (SG101 only) and three treated groups (SG101 + 0.1, 1, or 10 MOI of ΦSG-JL2).

Respiratory reproduction of fowl typhoid was performed as previous

(4). Briefly, five microliter from each tube was inoculated into each chick via intratracheal route and they were observed for mortality for 15 days after inoculation. After 15 days, surviving chicks were sacrificed to observe lesions on the livers (hepatic necrotic foci). The dead chicks were not included for counting of lesion-positive chicks. The surviving, lesion-negative chicks were used for the calculation of protection rate.

2.10. Statistical analysis

The Kaplan-Meier survival curves were drawn and the log lank test for the comparison between survival curves were performed using SAS (ver. 9.1.3). And also the protection rate of each group was evaluated via chi-square and Fisher's exact tests (95% confidence interval).

3. RESULTS

3.1. Host range of ϕSG-JL2

ϕSG-JL2 plated at efficiency of < 0.5 x 10^{-6} on *S.* Pullorum strain SP4, but at efficiency of < 6.5 x 10^{-9} on *Salmonella* Enteritidis, *Salmonella* Typhimurium, SG-9R (a rough vaccine strain of *S.* Gallinarum), and *E. coli*. Determination of the host range of ϕSG-JL2 using 106 strains of *S.* Gallinarum isolated in Korea between 1994 and 2006 demonstrated ϕSG-JL2 was lytic to 98.1% of the isolates, indicative of its utility in the identification of *S.* Gallinarum and for prophylactic application against fowl typhoid.

3.2. Morphology of ϕSG-JL2

Electron microscopy of negatively stained preparations of ϕSG-JL2 virions revealed hexagonal heads with a diameter of about 54 nm similar to other T7-like viruses (49) (Fig. 1).

3.3. One-step growth curve of ϕSG-JL2

A very short latent period (< 10 min) was evident, and burst-out of phage particles occurred between 10 and 15 min (Fig. 2).

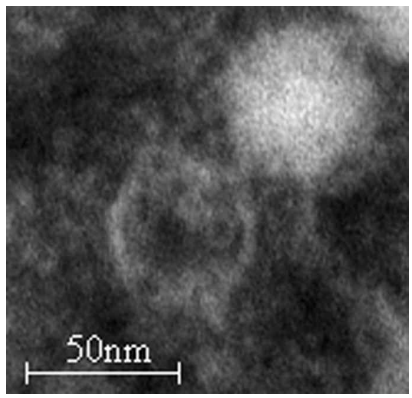

Fig. 1. Ultra microscopic structure of ϕSG-JL2 by electron microscopy

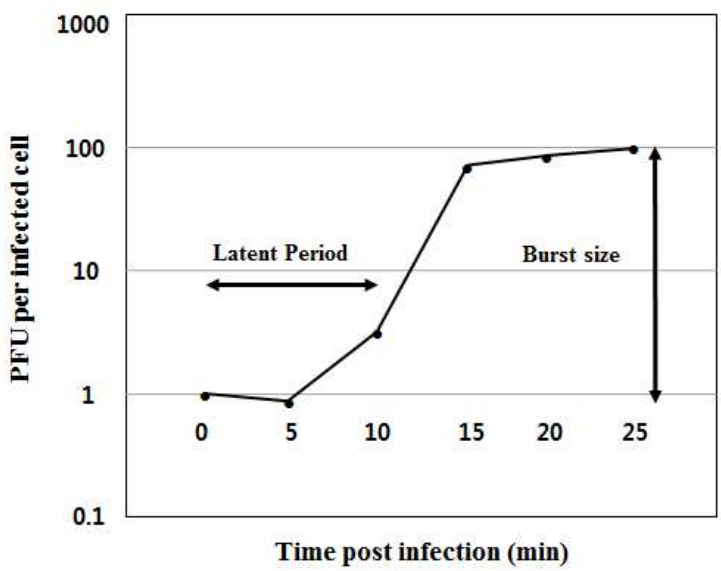

Fig. 2. One-step growth curve of ϕSG-JL2

3.4. Heat and pH susceptibility of ϕSG-JL2

PFU of ϕSG-JL2 was slightly decreased from 2 x 10^9 to 1.5 x 10^9 and 1 x 10^9 at 55℃ for 30 and 60 min, respectively, but CFU of host bacteria, SG002, decreased from 2 x 10^8 to 0. According to the pH susceptibility test, ϕSG-JL2 was completely inactivated just after mixing with pH 3.0 and pH 2.0 solutions and incubation for 10 min and it was highly susceptible to low pH condition.

3.5. Determination of the ϕSG-JL2 genome sequence

The ϕSG-JL2 genome was found to contain 38,815 bp of nucleotides and to possess an overall GC content of 50.9%. Fifty-five putative genes were identified in the same strand and functions were assigned to 30 genes based on the high amino acid similarity with known proteins (Table 1).

Table 1. Gene and protein identities of ϕSG-JL2 with ϕYeO3-12 and ϕT3 and other bacteriophages

Gene	Range	Length (aa)[a]	Mass (kDa)	pI	Ribosome binding site and initiation codon[b]	Identity (%)		Function
						ϕYeO3-12	ϕT3	
0.3	1050-1505	152	17.01	6.82	GAGGTaacaccaaAUG	99.3	98.0	S-adenosyl-L-methionine hydrolase
0.3B	1131-1505	125			GAGGTGaacAUG	98.4	97.6	
0.45	1730-1927	66			AGGActaacaccAUG	98.5		
0.6A	2105-2341	79			GGTGaaacacgcAUG	64.6	74.7	
0.6B	2105-2498	131			GGTGaaacacgcAUG	77.9		
0.7	2516-3622	369			AGGAcactgaacgAUG	87.3	91.9	Protein kinase
1	3696-6347	884	98.80	7.32	GAGGTaagcaAUG	99.2	99.0	RNA polymerase (RNAP)
1.05	6449-6955	169			GAGgtttactttAUG	17.8	18.3	gene 1.05 protein
1.1	7051-7188	46			GAGGtaagatactAUG	100	97.8	
1.2	7191-7466	92			GGAGtggaactaAUG	98.9	94.6	dGTP triphosphohydrolase inhibitor
1.3	7564-8577	338			GAGGaacaaccgtAUG	90.5	93.2	DNA ligase
1.5	8658-8732	25	2.82	3.32	AGGAGacacaccAUG	92.0	96.0	
1.6	8748-9002	85	9.83	11.18	TAAGGAGacaacatcAUG	98.8	97.6	
1.7	9005-9493	163			TAAGGAGGTtctgtaAUG	77.9	91.4	gene 1.7 protein
1.8	9483-9626	48			AGGggctgtgctAUG	89.6	89.6	
2	9616-9777	54			TAAGGAGGctcaaaGTG	65.4	94.4	Host RNAP inhibitor
2.5	9833-10528	232			AAGGAGaaacattAUG	99.1	98.7	Single-stranded DNA-binding protein
3	10531-10989	153	17.64	9.48	GAGGacttctaAUG	100	92.8	Endonuclease
3.5	10985-11437	151			AAGGAGtaaagaaaaAUG	98.0	96.0	Amidase (lysozyme)
3.7	11445-11549	35			GAGGgtgataccAUG	100	97.1	
4A	11619-13316	566			AAGGAatgtacaAUG	95.2	99.3	DNA primase/helicase
4B	11805-13316	504			AGGAGGcagcaagcctAUG	98.8	99.2	DNA helicase
4.15	11885-11989	35			AGGAGacAUG	94.3		
4.3	13416-13625	70			AGGAGacacatcAUG	100	97.1	
4.5	13641-13922	94			TAAGGAGcgcacactAUG	100	96.8	
5	13993-16104	704			AAGGAGGgcattAUG	97.4	87.4	DNA polymerase
5.5	16124-16426	101			TAGGAGaaacattAUG	100	52.5	Growth on lambda lysogen? H-NS inhibitor?
5.5-5.7	16124-16632	170	18.46	9.25	GGAGaaacattAUG	100	35.3	
5.7	16426-16632	69			GAGGTGttcaaAUG	100	88.4	
5.9	16632-16811	60			GGAGGTtgcgtAUG	98.3	21.7	recBCD nuclease inhibitor
6	16811-17719	303			GGAGGatgacgaAUG	98.0	78.2	Exonuclease

6.1	16848-17075	76			GGAGatgcGUG	97.4		
6.3	17704-17814	37			AAGGAGatttacttAUG	100	97.3	
6.5	17910-18152	81			GAGGTGAatttAUG	98.8	100	
6.7	18160-18408	83			AGGAGtaacgatAUG	98.8	100	Excreted head protein
7.3	18439-18756	106			GGAGaaacatcAUG	97.2	94.3	Tail protein (host specificity)
8	18770-20374	535			AGGAGGactgaAUG	99.6	98.7	Head-to-tail joining protein
9	20479-21408	310			AGGAGatttaacaAUG	99.0	95.2	Capsid assembly protein
10A	21568-22611	348			TAAGGAGattcaacAUG	98.3	97.7	Major capsid protein 10A
10B	21568-22745	393	41.78	6.47	TAAGGAGattcaacAUG	82.7	82.2	Minor capsid protein 10B
11	22830-23417	196			AGGAGGTaacatcAUG	99.5	99.5	Tail tubular protein A
12	23436-25838	801			AAGGAGGctctAUG	98.4	97.6	Tail tubular protein B
13	25914-26321	136			GGTtaaagcattAUG	97.1	96.3	Internal virion protein A
14	26327-26917	197			AGGAGGtaactAUG	99.5	98.0	Internal virion protein B
15	26923-29163	747			GGAGGTaataAUG	98.9	69.5	Internal virion protein C
16	29185-33144	1320	143.71	8.26	TAAGGAGGctccAUG	98.7	66.7	Internal virion protein D
17	33219-35192	658			AAGGAGGTcacAUG	31.9	33.8	Tail fiber protein
17.5	35206-35406	67			AGGAGGacataAUG	91.0	86.6	Lysis protein (Holin)
18	35413-35676	88			TAAGGAGtaacctAUG	98.9	72.7	DNA-packaging protein A
18.5	35769-36218	150	16.95	9.41	GGAGGTGttAUG	98.7	52.7	Endopeptidase; Lambda Rz homologue
18.7	35884-36135	84			AAGGAGGTaatccaaaAUG	98.8	47.6	Lambda Rz1 homologue
19	36196-37959	587			TAAGGAGatgcagaAUG	99.7	97.6	DNA-packaging protein B
19.2	36845-37075	77			AAGGAactcgaagataaccGUG	100	81.8	
19.3	37382-37507	42			GGTtccgcgAUG	100	95.2	
19.5	38203-38349	49			AAGGAGGTGgctcaAUG	98.0	95.9	

[a] aa, amino acids

[b] Lowercase letters indicate spacer nucleotides

3.6. Regulatory elements of ϕSG-JL2

Three major early promoters (A1, 460 - 489; A2, 589 - 618; and A3, 700 - 728) and a minor leftward promoter A0 (142 - 116) for host RNA polymerase (RNAP) were identified in the non-coding region near the left end of the ϕSG-JL2 genome. The nucleotide sequences of host promoters are exactly same as ϕYeO3-12 and T3 (A1), or identical only to ϕYeO3-12 (A0) or T3 (A2 and A3).

Altogether, 15 putative ϕSG-JL2 promoters were identified in the phage genome (Figs. 3 and 4), and most of them were similar in position and sequence to those of ϕYeO3-12 with slight nucleotide changes (ϕOL, ϕ1.1, ϕ1.5, and ϕOR). The consensus promoter sequence of ϕSG-JL2 is exactly same as those of ϕYeO3-12 and T3, but is apparently different from that of T7. The consensus sequence of the promoter opening site of ϕSG-JL2/ϕYeO3-12/T3 is similar to that of T7 (TAAA vs. TATA) and base compositions of ϕSG-JL2 of class II and class III promoters were similar to those of ϕYeO3-12 and T3.

The CJ(concatemer junction) terminator (5'-ATCTGTT-3') was located just after the LTR (231 to 237) and was conserved among T7-like viruses. A putative *rho*-independent early transcriptional terminator T_E for the host RNAP has been identified at positions 8,591 to 8,612, and the stem-loop structure (ΔG = -14.9 kcal/mol) and following U-tract (UUUCUU) are identical to that of T3 (50, 51). The T_E of ϕSG-JL2 locates immediately downstream of gene 1.3, as do those of ϕYeO3-12, T3 and T7 (19, 50, 51). A putative major

terminator, T_{ϕ}, has been identified just downstream of gene 10 at position 22,772 to 22,792, and the stem-loop structure (ΔG = -6.4 kcal/mol) and following U-tract (UUUUUU) are similar to that of ϕYeO3-12 (50, 51).

T3 and T7 RNAs are cleaved by the host enzyme RNase III at specific sites that form a stem and loop structure. Overall, 10 putative RNAse III sites analogous in their positions and sequences to those of ϕYeO3-12 and T3 phage have been identified in ϕSG-JL2 (50, 51); the sequences and the free energies are summarized in Table 2. R*0.3*, R*3.8*, and R*4.7* are identical to those of ϕYeO3-12 and T3, and R*13* is identical only to that of ϕYeO3-12. The nucleotide sequences of R*0.45*, R*1.3*, and R*18.5* are relatively variable among compared phages.

Table 2. Comparison of predicted RNase III sites of bacteriophage ϕYe-O3-12 and ϕSG-JL2

Name of putative RNase III site	Range	⊿G (kcal/mol)	Sequence of predicted stem-loop[a]
ϕYeO3-12 R*0.3*	956-1009	-17.6	UAAGCGAAUAACUCAAGGUCGCACUGAAAGCGUGGCCUUUAU/GAUAUUCACUUA
T3 R*0.3*	822-875	-17.6	----------/----------
ϕSG-JL2 R*0.3*	969-1022	-17.6	----------/----------
ϕYeO3-12 R*0.45*	1490-1545	-21.5	GUAAGUGUUAAACUCAAGGUC GCUCCAUGCGAGUGGCCUUUAU/GAUUAUCACUUAU
T3 R*0.5*	1359-1408	-20.8	...----------AU--A-G-----..----------/-----A--.--..
ϕSG-JL2 R*0.45*	1662-1717	-26.5	-------A--G---------- --A-UG--AU-U----------/----------
ϕYeO3-12 R*1*	3245-3293	-20.7	GAGUCUUUUCUUACAGGUCAUCAUGUGGUGGCCUGAAU/AGGAACGAUUU
T3 R*1*	2908-2956	-20.5	----------A----------/----------
ϕSG-JL2 R*1*	3628-3676	-20.9	-------A----------AUC----------/---U-------
ϕYeO3-12 R*1.1*	6340-6391	-20.4	GAGAGUUAAACUUAAGGUCAUCACCGACGGUGGCCUUUGU/GAUUAACUUUC
T3 R*1.1*	6003-6053	-20.4	----------A----------/----------
ϕSG-JL2 R*1.1*	6972-7022	-20.2	--------GC--C----------/----------
ϕYeO3-12 R*1.3*	6856-6896	-18.2	GAAUCCU/UAAGGUCACUU AACAUGAGUGGCCUUUGU/GAUUC
T3 R*1.3*	6519-6558	-16.9	-------/----------U-----..-------C--/-------
ϕSG-JL2 R*1.3*	7488-7527	-16.3	------/--------UC---U----UG----------/------
ϕYeO3-12 R*3.8*	11350-11377	-14.9	UAAAGGGAGACUUAACGGUUUCCCUUUG
T3 R*3.8*	10616-10642	-13.7	----------.----------
ϕSG-JL2 R*3.8*	11564-11591	-14.9	----------
ϕYeO3-12 R*4.7*	13706-13754	-21.7	AAGUGAUAAACUCAAGGUCGCCCAAGGGUGGCCUUUAU/GAUUAUCAUUU
T3 R*4.7*	12970-13018	-21.7	----------/----------
ϕSG-JL2 R*4.7*	13920-13968	-21.7	----------/----------
ϕYeO3-12 R*6.5*	17901-17971	-25.9	AAGUGAUAAACUCAAGGCUCUCUGUA UUAACCCUCACUAAAGGGAAGAGGGAGCCUUUAU/GAUUAUUACUU
T3 R*6.5*	17135-17206	-26.3	----------AC-A----------/----------
ϕSG-JL2 R*6.5*	17812-17882	-24.8	--------C---------- ----------/----------
ϕYeO3-12 R*13*	26211-26248	-21.0	GUCUCCCUGUGGUGAAUUAACCCUCACUAAAGGGAGAC
T3 R*13*	25443-25480	-19.9	---------C----------
ϕSG-JL2 R*13*	25848-25885	-21.0	----------
ϕYeO3-12 R*18.5*	36435-36488	-23.4	UAAGUGACAUACUCAAGGUUCUCCACUCGGGGGAGCCUUUAU/GGAUGUUAUUUG
T3 R*18.5*	35035-35085	-23.6	AC-----U--G--------.-----.UA--.--UG-------/--------C--GU
ϕSG-JL2 R*18.5*	35677-35729	-23.5	----------.--------/----------

a Slashes indicate cleavage sites; periods indicate deletions; homologous nucleotides are represented by dashes

3.7. Origins of DNA replication

The putative primary replication origin of ϕSG-JL2 DNA (R) (Fig. 3) could be tentatively placed at position 6,363 - 6,614 between genes 1 and 1.05, slightly different from those of ϕYeO3-12, T3, and T7. The counterparts of T7 ϕOL and ϕOR promoters which were proposed to be secondary origins of replication (19) were found in the ϕSG-JL2 genome; they contained A+T rich regions (334 - 338) and primase sites (38111 - 38115).

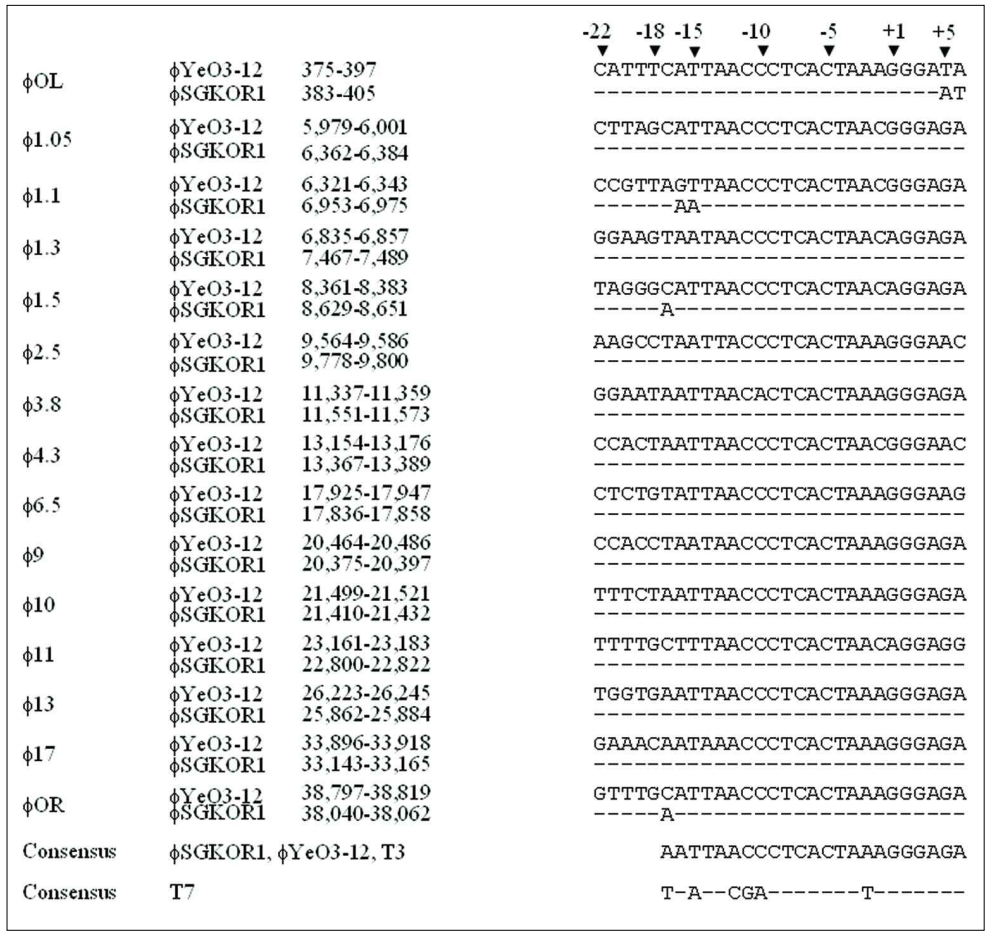

Fig. 3. Comparison of ϕSG-JL2 (ϕSGKOR1) and ϕYe-O3-12 promoters. The 15 putative promoter sequences of ϕSG-JL2 are aligned with those of ϕYeO3-12. The positions of the first nucleotides of the promoter sequences in the phage genome are given. Homologous nucleotides are represented by dashes.

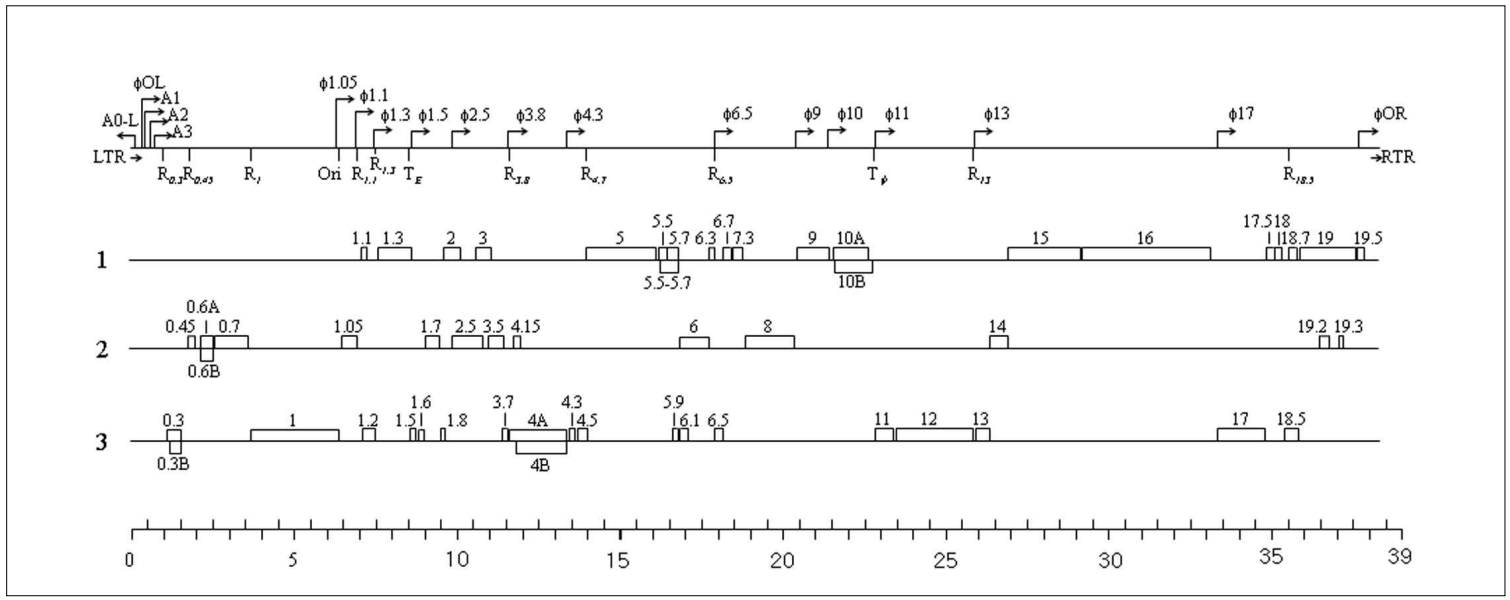

Fig. 4. Putative genome organization of ϕSG-JL2. The locations of putative regulatory elements, host (A0 to A3) and phage (ϕL to ϕR) promoters, RNase III recognition sites (R0.3 to R18.5), terminators (T_E and T_ϕ), and replication origin (Ori) are represented at the top, and the predicted ORFs are numbered and arranged according to reading frame (1, 2, and 3). A point on the scale represents 0.5 kb.

3.8. Genome ends of ϕSG-JL2

The left end noncoding region of the ϕSG-JL2 genome contains the LTR; CJ (concatamer junction, 231 - 237) terminator that is sensitive to lysozyme-mediated RNAP instability; repeats of short sequences (16 repeats of CCTAAAG and single nucleotide variants); an A+T rich region (361 - 390, 68.5%) that contains the ϕL replication origin; the A1, A2, and A3 promoters for host RNAP; the R*0.3* RNase III cleavage site; and the start of the coding sequence of gene 0.3 (19, 50). The right end of ϕSG-JL2 DNA contains the RTR, repeats of short sequences similar to that found near the left end (12 repeats of CCTAAAG and single nucleotide variants); coding sequence of gene 19.5; an A+T rich region (38008 - 38167, 65%) that contains the ϕR replication origin; and the end of the coding sequence of gene 19. The 230 bp terminal repeats (LTR and RTR) are 94.4% and 91.4% identical, respectively, to those of ϕYeO3-12 and T3, and the length is similar to T3 (231 bp) and ϕYe-O3-12 (232 bp) (50, 51).

3.9. Other features of the nucleotide sequence

The recognition sites of DNA cytosine methyltransferase (Dcm) and DNA adenine methyltransferase (Dam) were found to be infrequent in the ϕSG-JL2 genome (Table 3), but one Dam site was located upstream of 0.3 gene.

The recognition sites of type I restriction enzymes, *sty*SBI and *sty*SBLI, in ϕSG-JL2 genome occur only once and three times, respectively, and are distant from gene 0.3, at 23,718 and 10,375,

respectively. Only the frequencies and locations of *sty*SBI and *sty*SBLI recognition sites in ϕYeO3-12, T3, and T7 apparently contrasted with those of ϕSG-JL2 (Table 3).

A type III R-M enzyme, *Sty*LTI is encoded by chromosomal genes of *S.* Typhimurium LT7 and recognizes the sequence CAGAG and methylates the second adenine in one strand (16). The frequencies of CAGAG were different (11 vs. 79, respectively) in the ϕSG-JL2 genome, and CAGAG appeared first far downstream (14,056) from the 0.3 gene compared with other phages (Table 3).

The genomic nucleotide sequence of ϕSG-JL2 was compared with those of ϕYeO3-12 and T3. Synteny plots revealed that, similar to ϕYeO3-12, the genome sequence of ϕSG-JL2 was dissimilar to T3 in two distinct regions, genes 5 to 6.1 and genes 15 to 18.7 (Fig. 5).

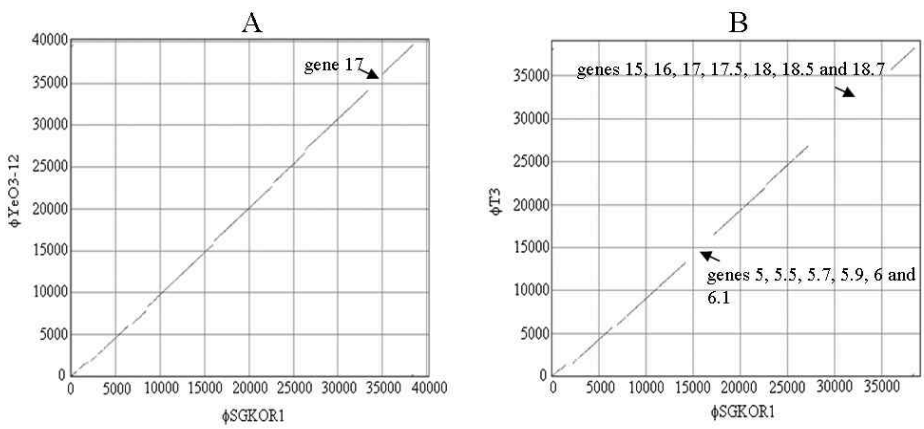

Fig. 5. Comparison of gene contents of ϕSG-JL2 with those of ϕYeO3-12 and T3

Table 3. The frequency of restriction enzyme and methylase recognition sites in the genomes of ϕSG-JL2, ϕYeO3-12 and T3

R-M system/ Families	Enzyme	Recognition sequence	No. of recognition site (the location of the first recognition site)			
			ϕSG-JL2 (1,050-1,505)[a]	ϕYeO3-12 (1,035-1,490)	T3 (901-1,359)	T7 (925-1,276)
Methylase	Dcm	CC(A/T)GG	0	0	2 (19,053)	2 (2,366)
Methylase	Dam	GATC	5 (921)	3 (7,382)	10 (2,380)	6 (8,312)
Type I/A	*Eco*KI	AAC(N)$_6$GTGC	7 (3,383)	5 (3,110)	4 (5,490)	4 (15,161)
Type I/A	*Sty*SBI	GAG(N)$_6$RTAYG	1 (23,718)	2 (7,562)	2 (3,295)	7 (1,491)
Type I/A	*Sty*SPI	AAC(N)$_6$GTRC	8 (3,383)	9 (3,000)	7 (2,663)	9 (6,356)
Type I/B	*Eco*AI	GAG(N)$_7$GTCA	6 (9,106)	5 (8,232)	5 (8,162)	0
Type I/B	*Sty*SKI	CGAT(N)$_7$GTTA	2 (3,953)	2 (3,570)	1 (3,233)	0
Type I/D	*Sty*SBLI	CGA(N)$_6$TACC	3 (10,375)	6 (5,091)	7 (4,754)	13 (2,107)
Type III	*Eco*P15I	CAGCAG/CTGCTG	4 (9,724)/40	4 (8,543)/38	5 (1,938)/47	0/36
Type III	*Sty*LTI	CAGAG/CTCTG	11(14,056)/78	13 (12,520)/72	14 (4,795)/63	15 (2,287)/72

[a] Location of gp0.3 (SAMase)

3.10. Translational features of ϕSG-JL2

Just as other T7-like viruses, the ϕSG-JL2 genome is highly packaged and the coding region covers 90% of the genome, which is slightly lower than T3 (91%) and ϕYeO3-12 (92%) (47). Presently, the gene content of ϕSG-JL2 was found to be similar to ϕYeO3-12 and T3, and the identities of the putative ϕSG-JL2 proteins ranged from 17.8% - 100% compared to those of ϕYeO3-12 and T3 (Table 1).

The initiation codon for gp2, gp6.1, and gp19.2 was GUG but all other genes started with AUG. Preferred stop codons were UAA (69.1%) and UGA (29.1%). It has been shown that all predicted genes are preceded by a potential SD sequence of 3 - 10 nucleotides capable of uninterrupted pairing with nucleotides near the 3' end of 16S rRNA (3'-AUUCCUCCACUAG) (62, 66). The use of GCU (alanine) as the second codon in highly expressed genes of T7 and ϕYeO3-12 is also observed in comparable genes of ϕSG-JL2 (50).

The ribosomal +1 and -1 frameshifts during translation of genes 0.6A, 5.5, and 10A in T7 generate gp0.6B, gp5.5-5.7, and gp10B (13, 18). The nucleotide sequences of 0.6A and 5.5 frameshifting regions of ϕSG-JL2 were observed to be exactly the same as those of ϕYeO3-12 but were different from those of T7. Although the experimental data on frameshiftings in 0.6A and 5.5 of ϕYeO3-12 are unavailable, the putative gp0.6B and gp5.5-5.7 of ϕSG-JL2 are listed in Table 1. In T7 and T3, overlapping valine-phenylalanine and proline-lysine codons by -1 frame, respectively, near the stop codon of 10A render base pairing of corresponding tRNAs with -1 frame

codons, and the hypothetical pseudoknots may enhance the ribosomal frame shifting (13 - 15). As observed with T3 and ϕYeO3-12, ϕSG-JL2 shared the same 10A motif for frame shifting.

Homing nuclease is contained in a group I intron and functions in site-specific gene conversion of the group I intron by catalyzing double-strand breaks in the recipient target site (18). Relative to ϕSG-JL2, ϕYeO3-12 acquired genes 1.45, 4.2, 5B, 5.3, and 13.5, and among them genes 1.45, 5.3, and 13.5 represent putative group I introns or homing endonucleases grouped into the ββα - Me family (31, 50, 51). The homing endonucleases are common in other T7-like viruses such as T3, T7, ϕYeO3-12, ϕA1122, ϕgh-1, ϕVpV262, and ϕKMV, but the copy numbers vary from 1 - 4 (20, 21, 26, 36, 50, 51). The origins of homing endonucleases have been unclear, but lack of known homing endonucleases homologs in the ϕSG-JL2 genome reflects the relatively low rate of genetic exchanges with genetic pools containing homing endonucleases during its evolution.

Holins are grouped into two classes on the basis of the number of transmembrane domains. Class I holins have three transmembrane domains and class II holins have two transmembrane domains (80). The holins of ϕYeO3-12, T3, and T7 are predicted to have two transmembrane domains and so represent class II holins (49, 80), but our present analysis using Ver. 2.0 of the TMHMM program (27) revealed only one transmembrane domain in the holins of ϕSG-JL2, ϕYeO3-12, T3, and T7, and charged N-termini and C-termini in the periplasm and cytosol, respectively. In view of the accurately

predicted transmembrane domains of other class I (λS and Hol500) and class II (λ21S and HolTW) holins (39, 40, 80) by the program, and the presence of dozens of holins containing single transmembrane domain in the GenBank (accession nos. NP_795652, YP_238508, AAM83087, YP_001333670, CAC17008, BAD51461, NP_813783, AAD04658, NP_043494, NP_536830, AAQ75055, CAK25980, YP_001522836, YP_655476, CAA81341, YP_001468955, NP_839939, YP_399007, NP_853599, NP_700424, YP_003932, NP_803401, ABF72775, NP_795484, NP_795705, NP_268941, ABF31779, ABF33660, YP_001430016, CAB52539, YP_025044, AAP42307, YP_001671761, CAC48115, NP_835573, YP_908848, YP_001469228, NP_061647, YP_803187, NP_891825, and AAX11974), assignment of a new class to the holins of T7-like phages should be considered.

3.11. Identification of proteins involved in host adaptation of ϕSG-JL2

Among the proteins with known functions, non-structural proteins (gp0.3, gp0.7, gp1, gp1.2, gp1.3, gp2, gp2.5, gp3.5, gp4A, gp4B, gp5, and gp6) and host specificity-related proteins gp17 (tail fiber) and gp7.3 (tail protein) were targeted for polymorphism analyses among ϕSG-JL2, ϕYeO3-12, and T3. We computed Pi[a]/Pi[s] ratios of the target genes with the DnaSP program (window length, 50; sliding size, 10). The Pi[a]/Pi[s] ratios of genes 0.3, 1, 2.5, 3.5, 4B, and 7.3 ranged from 0.033 - 0.059, but those of genes 0.7, 1.2, 1.3, 2, 4A, 5, and 6 ranged from 0.094 - 1.264. The Pi[a]/Pi[s] ratio of gene 2

exceeded 1, indicative of positive Darwinian selection (Table 4).

The genes 0.7 and 6 possessed local polymorphic regions of which Pi[a]/Pi[s] ratios exceeded 1 (Table 4).

Gp17 is a tail fiber protein that attaches to a host receptor and determines host specificity. The conserved N-terminal of T7 gp17 interacts with a head-tail connector protein and the hypervariable C-terminus interacts with host receptor (71). The amino acid similarities of gp17 among the compared phages are only 30.1% - 33.8%.

Table 4. The Pi[a]/Pi[s] ratios of ϕSG-JL2, ϕYeO3-12 and T3 genes

Gene	Protein	Mean Pi[a]/Pi[s] ratio[a]	Variable region (Pi[a]/Pi[s])[b]
0.7	Protein kinase	0.155	757-777 (1.007)
2	Host RNA polymerase inhibitor	1.264	1-93 (1.724), 81-101 (1.685), 90-110 (4.126)
6	Exonuclease	0.241	337-357 (1.738), 364-384 (1.000), 373-393 (2.828)

[a] Window length, 50; sliding size, 10

[b] Region of whose Pi[a]/Pi[s] ratio is more than 1.000 (Window length, 21; sliding size, 9)

3.12. Prophylactic efficacy of ϕSG-JL2 against fowl typhoid in chickens

I applied the respiratory model system to test the prophylactic efficacy of ϕSG-JL2 against fowl typhoid. The untreated control group showed 85% (17/20) of mortality, but the treated groups which were treated with different moi (0.1, 1, and 10) of ϕSG-JL2 showed 5%, 10%, and 15% of mortalities, respectively (Fig. 3). The survival curves were significantly different between untreated and treated groups ($p < 0.05$). The protection rates of the untreated, 0.1, 1, and 10 moi treated groups were 10%, 70%, 80%, and 65%, respectively, and the difference between untreated and treated groups were significant ($p < 0.05$). The protection rates of the treated groups were insignificantly different each other ($p > 0.05$).

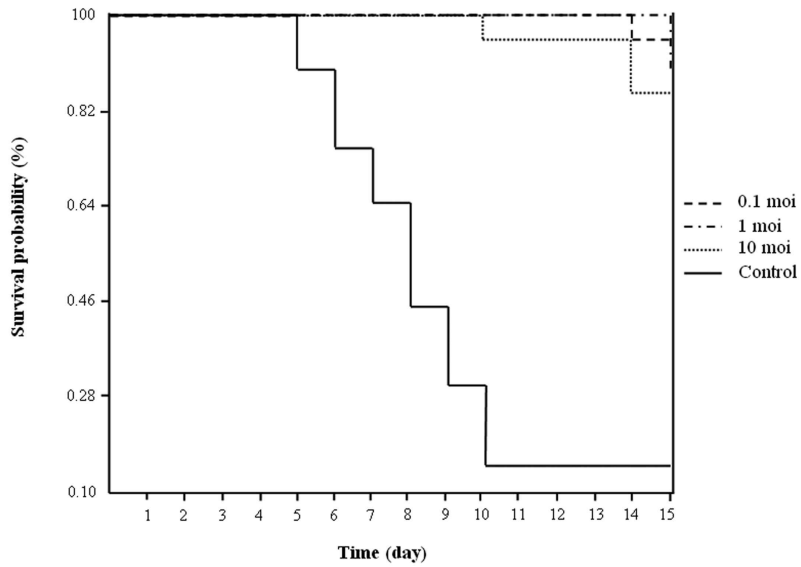

Fig. 6. Survival curves of ϕSG-JL2-treated and untreated groups. Eighty 13-day-old commercial male brown layer chicks were challenged with a field strain of serovar Gallinarum biovar Gallinarum (SG101) directly or after it was mixed with ϕSG-JL2 (MOIs, 0.1, 1, and 10) for 4 h at room temperature, and mortality was observed for 15 days.

4. DISCUSSION

The receptors of T7-like viruses have been reported to be lipopolysaccharide (LPS) but different phages bind different moieties of LPS (45, 54). Neither the T3 or T7 types of T7-like viruses form plaques on smooth *E. coli* strains, and binding occurs to glucose residues in the outer core (T3) and more inner moieties of LPS (T7) (45, 54). *Salmonella* phage SP6 grows on both rough and smooth strains, but ϕYeO3-12 is specific to the O3-antigen of *Yersinia enterocolitica* (1, 45). No plaque formation of ϕSG-JL2 occurs on SG-9R, which lacks LPS O-side chains (67), consistent with the participation of the O-antigen as the receptor.

In the study of one-step growth curve, a very short latent period (<10min) of ϕSG-JL2 was evident. The overall one-step growth cycle was slightly shorter than that of ϕYeO3-12 but the burst size (about 100 pfu per infected cell) was similar to that of ϕYeO3-12 (49).

According to the pH susceptibility test, ϕSG-JL2 was completely inactivated at pH of gastric juice. Thus, when ϕSG-JL2 is treated via oral route anti-gastric juice compound should be mixed together with the phage for better prophylactic efficacy.

According to the genome analysis, the GC content of ϕSG-JL2 is slightly higher than T7 (48.4%) but similar to ϕYeO3-12 (50.6%) and T3 (50.0%) (19, 50, 51). ϕSG-JL2 shows no significant similarity with

other *Salmonella* phages compared in terms of amino acid similarity of gene contents.

The T7 promoter (-17 to +6) has three distinct elements: RNAP binding (-17 to -6), promoter opening (-4 to -1), and initiation and elongation sites (+1 to +6) (2, 19, 23, 56, 77). Mutations in the RNAP binding site decrease the affinity of RNAP and bases in the region interacting with amino acid residues of the RNAP (11, 12). The 93 - 101 and 739 - 770 residues contacted the -17 to -13 and -11 to -7 regions of the T7 RNA promoter, respectively. Comparison of amino acid residues of ϕSG-JL2 in the regions revealed 100% (739-770) or high (93-101) similarity to those of ϕYeO3-12 and T3, but apparent differences from those of T7. The transcription efficiency of T7 RNAP can be apparently decreased by mutations (A to C at -10 or C to A at - 12) and even abrogated by a G to C mutation at -11 in the T7 promoter (25). Therefore, high nucleotide variations between ϕSG-JL2/ϕYeO3-12/T3 and T7 in the -17 to -7 region of the consensus sequences may be the result of co-evolution of the RNAP and promoter, which results in phage-specific promoter recognition (Fig. 2). The selection of the transcription start site in the T7 promoter is determined by H784 of T7 RNAP (7), and the presence of H785 and similar amino acid residues around it in RNAPs of ϕSG-JL2, ϕYeO3-12, and T3 might be related to identical consensus sequences of the initiation and elongation sites between ϕSG-JL2/ϕYeO3-12/T3 and T7. The stronger activity of the class III T7 promoters are linked to an A+T rich region without interruption

of G or C nucleotides between -22 and -18. It increases affinity of the T7 RNAP (74), but presently there were no such evident differences between class II and class III promoters of ϕSG-JL2, ϕYeO3-12 and T3 (Fig. 2). The A+T rich recognition loop of T7 RNAP consists of amino acid residues from 93 to 101, and K93 and K95 are suspected to interact with the A+T rich region (74). The RNAPs of ϕSG-JL2, ϕYe-O3-12, and T3 have the same (K95) and different (A93) residues, therefore, they may recognize class II and class III promoters differently than does T7 RNAP.

The T7 primary replication origin is located between the noncoding region of gene 1 and gene 1.1, and is characterized by two phage promoters (ϕ1.1A and ϕ1.1B), a high A+T rich region, and a primase site (T7-type: 5'-GACCC-3') that can initiate rightward leading strand synthesis (61). The primary replication origins of ϕYeO3-12 and T3 have been mapped downstream of gene 1 overlapping the 5' end of gene 1.05 and they include a phage promoter, ϕ1.05, a putative stem and loop sequence (5'-GGGAGACtacttaagGTCTCCC-3'), and an A+T rich region containing a primase site (T3 type: 5'-GACAC-3') near the stem and loop sequence (50, 62). ϕSG-JL2 had a 12-nucleotide deletion just after the stem and loop sequence that resulted in the loss of the primase site in the A+T rich region (78.3%, 6401 - 6460). The first T7-type primase site appeared downstream (6610 - 6614) of the A+T rich region. The primase-helicase of T7 binds randomly to single-stranded DNA and then translocates in a 5'-to-3' direction until it reaches the priming

signal (61). Thus, the putative primary replication origin of ϕSG-JL2 DNA (R) was tentatively placed at position 6,363 - 6,614 between genes 1 and 1.05, slightly different from those of ϕYeO3-12, T3, and T7. The T7 ϕOL and ϕOR promoters are proposed to be secondary origins of replication (19). Counterparts to both promoters were found in the ϕSG-JL2 genome; they contained A+T rich regions (334 - 338) and primase sites (38111 - 38115).

Restriction and modification (R-M) of foreign DNA by bacteria is a basic defense mechanism and phages have evolved to evade the host R-M system. Genome analysis of ϕYeO3-12 has revealed the markedly less frequent methylation of GATC and CC(A/T)GG by DNA cytosine methyltransferase (Dcm) and DNA adenine methyltransferase (Dam), respectively (42, 50). Furthermore, ϕYeO3-12 and T3 genomes are not methylated because S-adenosyl-L-methionine hydrolase (SAMase) degrades the methyl group donor in the host, and because almost all recognition sites are present downstream of gene 0.3 encoding SAMase (Table 3) (19, 70). Just like ϕYeO3-12, Dam and Dcm recognition sites were found to be infrequent in the ϕSG-JL2 genome (Table 3). Considering the high processing activity of Dam the recognition site can be methylated before SAMase translation, but the low copy number and localized presence of Dam in the replication site of bacterial genomic DNA may explain the normal replication of ϕSG-JL2 in *S.* Gallinarum (78).

T3 and T7 genomes were also resistant to the type I restriction enzyme *Eco*K1 (28, 29, 72, 73) because of SAMases and downstream

locations of the first recognition sequences, 5,490 - 5,502 and 15,161 - 15,173, respectively, from gene 3 (Table 3). Four *Salmonella* type 1 restriction enzymes (*sty*SBI, *sty*SPI, *sty*SKI, and *sty*SBLI) have been identified in *S.* Typhimruium, *S.* Potsdam, *S.* Kaduna, and *S.* Blegdam, respectively (46, 75, 76). The very low frequency and distant location from gene 0.3 of *sty*SBI and *sty*SBLI sites in ϕSG-JL2 genome may support the resistance of ϕSG-JL2 to the type I restriction enzymes just as T3 and T7.

A type III R-M enzyme, *Eco*P15, methylates the second adenine of CAGCAG sequence but recognizes two CAGCAG sequences in the inverse orientation for restriction (44). The resistance of T7 and susceptibility of T3 to *Eco*P15 restriction can be explained by the absence and multiple presences of the inversed sequences, respectively (Table 3) (63). *Sty*LT1 is a type III R-M enzyme and is encoded by chromosomal genes of *S.* Typhimurium LT7 (16). The enzyme recognizes the sequence CAGAG and methylates the second adenine in one strand, but whether it requires two inverse recognition sites or not is unclear. The frequencies of CAGAG and CTCTG sequences were different (11 vs. 79, respectively) in the ϕSG-JL2 genome, and CAGAG appeared first far downstream (14,056) from the 0.3 gene (Table 3). Therefore, the strand bias of CAGAG may support the hypothesis that *Sty*LT1 recognizes two inversed recognition sites just as *Eco*P15, but further study is required to understand biological meaning of the location bias of CAGAG in the ϕSG-JL2 genome.

The gp2 of T7 is reported to inhibit host RNAP by interaction with a dispensable region of β' subunit, and mutants carrying E1158K or E1188K mutations in *rpoC* are resistant to T7 (47). T3 productively infects a mutant carrying E1188K mutation, therefore, gp2 of T3 may interact with a different site of host RNAP from gp2 of T7 (8, 47).

The reasons and functions of local polymorphisms of gp0.7 and 6 are unclear, but they can be explained in part by evolution for optimal interaction with host proteins. Gp0.7 is a serine/threonine protein kinase and phosphorylates translational components (IF1, IF2, IF3, elongation factor G, and ribosomal proteins S1 and S6), host RNAP β' subunit, and enzymes related to mRNA metabolisms (RNase III and RNase E), resulting in exclusive phage gene expression (41, 43, 48, 55, 57, 58, 65, 81). Gp6 is an exonuclease and contributes to packaging concatemerized phage DNA by suppressing the packaging of host DNA (69). To date, interaction of gp6 with host proteins has been unknown, therefore, the reasons why gp6 possesses polymorphic regions need to be resolved.

During the early phase of evolution of an organism, horizontal genetic transfer may play a key role, but when it crosses the "Darwinian Threshold," vertical genetic changes become more important (26, 79). T7 group phages have been proposed as descendants of an ancient species that has crossed the "Darwinian Threshold" because of severely limited horizontal genetic exchange and conservation of essential genes and their layout (26). The

comparison of closely related phages, ϕSG-JL2, ϕYeO3-12, and T3, in the present study also revealed conservation of essential genes and their layout, but the presence of species-specific genes, especially gene 2, which may play key roles during host adaptation (47). Therefore, ϕSG-JL2 and variable genes identified in the present study may be useful for understanding vertical evolution of a phage during its adaptation to a specific host.

To date prophylactic or therapeutic phage therapies against *Salmonella* Typhimurium, *E. coli*, and *Bacillus anthracis* have been reported (5, 30, 64, 68), but phage therapy against *S.* Gallinarum has been rare. Although the high susceptibility of ϕSG-JL2 to low pH can be a drawback for oral treatment because of gastric acid, but mixture with acid-neutralizing reagents or direct spray of phage solution to chickens, floor, and environment may improve prophylactic efficacy of ϕSG-JL2. To control the fowl typhoid "test and slaughter" of positive flock has been the best policy, but in countries where the fowl typhoid is enzootic prophylactic application of bacteriophage can be one of measures to reduce horizontal transmission of multi-drug resistant *S.* Gallinarum between chickens, flocks or farms. Therefore, further studies to verify preventive efficacies of ϕSG-JL2 in various conditions which simulate the field conditions may be valuable to minimize economic losses caused by the fowl typhoid and antibiotics use.

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국문초록

Salmonella Gallinarum에 특이적인 T7-유사 용균 박테리오파지(ΦSG-JL2)의 특성 분석

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ΦSG-JL2는 조면 균체를 갖는 가금티푸스 백신주인 SG 9R, *Salmonella* Enteritidis, *Salmonella* Typhimurium, 추백리 원인균인 *Salmonella* Pullorum에 대해서는 용균을 일으키지 않으나 가금티푸스의 원인균인 *Salmonella* Gallinarum은 특이적으로 용균하는 박테리오파지로 유전체 구조, 비교 유전체학 및 가금티푸스 예방 효능 연구가 이루어지지 않아 본 연구를 수행하였다. ΦSG-JL2의 유전체는 38,815bp (GC 함량 50.9%; 230bp terminal repeats)로 55개 유전자가 동일 가닥의 DNA에 존재하였고, 이 중 아미노산 서열의 상동성을 비교 분석한 결과 30개 유전자의 기능을 확인하였다. 추정된 단백질 대부분의 아미노산 서열 상동성(tail fiber 31.9% 제외)과 유전체 구성이 *Yersinia enterocolitica*에 특이적인 ΦYeO3-12와 유사하였으나 *Salmonella* Gallinarum에 대한 숙

주특이성으로 신규한 T7-유사 박테리오파지로 분류하였다. 숙주 적응과 관련된 유전자 동정을 위해 아미노산 서열의 변화를 초래하는 염기서열 변이와 그렇지 않은 변이의 비율(Pi[a]/Pi[s])을 조사한 결과 RNA 중합효소를 코딩하는 gene 2가 관련된 것으로 분석되어 향후 박테리오파지의 숙주 적응 연구에 유용할 것으로 사료되었다. 또한 *Salmonella* Gallinarum 야외주를 기관 내 공격접종 시 ΦSG-JL2를 처리하는 경우 세균과 박테리오파지의 비율이 0.1인 경우에도 가금티푸스에 의한 폐사를 대조군 대비 유의적으로 예방하여($P<0.05$) ΦSG-JL2는 가금티푸스에 의한 경제적 피해와 항생제 남용을 줄일 수 있을 것으로 평가되었다.

Key words: T7 유사 바이러스, 가금티푸스균, 유전체 분석, 숙주적응, 예방법

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