Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 71-78

Genomic sequence analysis reveals new bacteriophage genome JH2

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ARTICLE INFO

Recieved: 03 October 2023

Accepted: 04 December 2023

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Keywords:

Bacteriophage
Enterotoxigenic *E. coli*
Staphylococcus phage
Genome sequence homology
JH2

ABSTRACT

In this study, an *E. coli* phage JH2 was isolated from swine feces in an industrial farm. Bacteriophage JH2 could carry 85% (n= 32) O8 ETEC (Enterotoxigenic *Escherichia coli*) strains when 81 alike-strains from Vietnam and China were tested. The genome size could be as large as JH2 had 77187 bps, a linear, double-stranded and CG = 38.89%. It shared a height similarity of 94.9% to *Staphylococcus* phage SA1 of GU169904 with 147303 bps, which is about double the size. JH2 carried 22 tRNA and no gene for known toxins. Under the electronic microscope the phage presented icosahedral heads, necks, contractile and long tails, and it belonged to the *Myoviridae* family. The phage demonstrated high stability to the physical and chemical factors, thermal sensitivity at 60°C and acid and alkaline resistance (pH= 5-9). The genome of JH2 contained 131 putative open reading frames (ORFs). One hundred and twenty-six ORFs (out of 131) were predicted and 121 ORFs had putative functions. From database, 22 tRNAs were predicted in the JH2 genome. *Staphylococcus* phage SA1 contains an almost complete phage genome of JH2 phage. The complete genome sequence of JH2 was analyzed. Comparing the complete genome sequences of JH2 and *Staphylococcus* phage SA1 showed 94.9% similarity.

Introduction

Porcine post-weaning diarrhea (PWD) occurs sporadically or as major outbreaks resulting in economic losses to the porcine industry (Do *et al*., 2006; Vidotto and One, 2009; Christine *et al*., 2010). ETEC (Enterotoxigenic *Escherichia coli*) strains that cause this disease are typically reduced F4 (K88) or F18 fimbriae and belong to a small number of O serogroups, with O8 being the most commonly reported worldwide (Han *et al*., 2007). Strains of this serogroup were responsible for recent exceptionally severe outbreaks of disease in pigs in North Vietnam, and almost all were O8:F4 ETEC or O8:F-: ETEC (Do *et al*., 2006). Antibiotics have been commonly used in the treatment of infectious diseases, but their widespread and improper use has led to antibiotic resistance to porcine colibacillosis (Lu and Koeris, 2011). Since antibiotic resistance is common among ETEC, these strains and the associated problem persist, such as ineffective vaccination treatment and difficulties with feed additives. It was thus decided to investigate the use of bacteriophage for prevention and therapy (Jamalludeen *et al*., 2008). Recently, scientists have reported on the isolation and application of phage in the treatment of animals with resistant *Escherichia coli* (*E. coli*) infections (Thung *et al*., 2018; Zhao *et al*., 2020). The goal of this study was to isolate and characterize the genomic sequence of bacteriophage and analyze the sequence data of bacteriophage lysis O8:F4 ETEC strains. The long-term implication is that the treatment and prevention of swine diseases is more efficient and the swine farming economy proportionally improves.

Materials and methods

Bacteriophage isolation and propagation

Fecal and sewage samples collected from the swine farm in Nanjing, China in 2011 were used to isolate Bacteriophages. Six *E. coli* isolates (VN1-O8: F18 ETEC, VN2- O8: F4 ETEC, VN3-O8:F4 STEC, VN4-O8:F18 ETEC, VN5-O8 ETEC, and VN6-O8 ETEC) from swine in Vietnam was inoculated with a mixture of equal proportions of the strains in the LB broth for 5 hours at 37°C. The samples (the fecal sample mixed in TS buffer, or sewage samples) were centrifuged before filtering through a membrane filter (Φ 0.45 µm) to remove impurities and bacteria. To enrich *E. coli* bacteriophages, 20 mL of LB broth, and 20 mL of a suspension of *E. coli* strains in broth culture (OD600 = 1.4) and sample were added to the flask and incubated at 37°C for 24 hrs with shaking. After incubation, the culture was centrifuged twice at 4,000 × g for 15 minutes at 4°C. The supernatant was collected into a new sterile flask and filtered through a sterile membrane filter ((Φ 0.45 µm). To detect the presence of phage in the filtrate, spot testing was performed as described by Kropinski *et al*. (2009). The characteristics of the phages obtained were the same as described by Jamalludeen *et al*. (2009a).

Electron microscopy

Phage preparations were applied to a carbon film and fixed to a copper grid negatively stained with phosphotungstic acid (PTA, 2% w/v). Phage morphology was examined using a transmission electron micro-

ISSN: 2090-6277/2090-6269/

scope (H_7650, Hitachi, Japan). Both phage morphology and dimension (capsid diameter, tail length) (Bai *et al*., 2013) were examined.

Host range assay

To investigate the host range of the phage JH2, six *E. coli* (VN1-O8: F18 ETEC, VN2- O8: F4 ETEC, VN3-O8:F4 STEC, VN4-O8:F18 ETEC, VN5-O8 ETEC, and VN6-O8 ETEC) isolates from swine in Vietnam (Hoa *et al*., 2013); twenty-two *E. coli* (named NJ1-22) isolates from swine farms in Nanjing, China; eight *E. coli* strains (NT1-O2 ETEC, NT2-O5 ETEC, NT3-O9 ETEC, NT4-O11 ETEC, NT5-O138 ETEC, NT6-O139 ETEC, NT7-O141 ETEC, and NT8-O149 ETEC) from the Institute Veterinary Research and Development of Central Vietnam; twenty *E. coli* isolates from bovine (JV1-20); and twenty-one *E. coli* isolates from chicks (LYT 15-36) (Hoa *et al*., 2013) were used to tested as described by Jamalludeen *et al*. (2009a).

One-step growth curve

The one-step growth assay was carried out as described previously (Bai *et al*., 2013). Five milliliters of LB broth were inoculated with 1% of a freshly grown VN2 culture 2×10^8 cfu/mL and 1% phage lysate 2×10^7 fu/mL. Incubation at 37°C was continued under aerobic conditions in a shaking incubator (240 rpm) for 15 minutes. The culture was diluted by at least 104-fold after infection then 1 mL sample was added to 20 mL LB broth and continued incubation at 37°C. After 5, 10, 15, 20, 25, and 30 minutes, then every 15 minutes infectious phage titer of the supernatant was enumerated by the plaque assays as described by Kropinski *et al*. (2009).

Extraction of DNA bacteriophage

Bacteriophage DNA was extracted as described previously (Sambrook *et al*., 1989; Pickard, 2009). Bacteriophage was allowed to completely lyse host *E. coli* strains in a soft agar overlay. SM buffer was added to the overlay and bacteriophages were allowed to diffuse into the buffer at 4°C for 3-4 h with gentle shaking. After the suspension was centrifuged at 4000 × g for 15 minutes, the supernatant was collected. Solid NaCl was added to the supernatant with a final concentration of 1 M followed by swirling. After incubation on ice for 1 hour, the suspension was centrifuged at 10,000 × g for 10 minutes at 4°C (Beckman Coulter, J2-MC Centrifuge). Polyethylene glycol (PEG 8000) was added to the supernatant with a final concentration of 10% (w/v), and the mixture was stirred slowly at room temperature. After standing for 1 hr on ice, the mixture was centrifuged at 10,000 × g for 30 minutes at 4°C. The bacteriophage pellet was then resuspended in 1 mL of SM buffer. An equal volume of chloroform was added to the phage suspension and underwent vortexing for 30 seconds. The phases were separated by centrifugation at 3,000 × g for 15 minutes at 4°C, and the aqueous phase was recovered. Pancreatic DNase I and RNase I were added to the aqueous phase with a final concentration of 5 and 1 µg/mL, respectively. The mixture was incubated at 37°C for 30 minutes. EDTA (pH = 8.0) was added to the mixture with a final concentration of 20 mM. Proteinase K was added to the mixture with a final concentration of 50 µg/mL and incubated for 30 minutes. Then sodium dodecyl sulfate (SDS, 10%) was added to the incubation with a final concentration of 0.5%, and the mixture was inverted several times prior to incubation at 56°C for 2 h. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v) was mixed into the sample. The aqueous phase was collected after centrifugation at 10,000 × g for 10 minutes and extracted with an equal volume of chloroform: isoamyl alcohol (24:1, v/v). Centrifugation was repeated, and the aqueous phase was collected. Two volumes of ice-cold 95% ethanol were added, and the aqueous phase was kept at room temperature for 20 minutes. The precipitate was collected by centrifugation at 10,000 × g for 10 minutes at 4°C and washed with cold 70% ethanol. Following centrifugation at 10,000 × g at 4°C for 30 minutes, the pellet was air-dried and dissolved in 20-35 µL TE buffer (10 mM Tris, 1 mM EDTA, pH = 8.0).

SDS-PAGE of JH2 phage particles

Bacteriophage particles by centrifugation through a glycerol step gradient were determined by Sambrook *et al* (1989). Afterward, purified particles were subjected to SDS-PAGE on precast 4-15% gradient TRIS acrylamide gels (BioRad) along with protein molecular weight markers. The phage suspensions (approximately 1010 cfu/mL) were boiled for 5 minutes and separated by SDS-PAGE loading buffer (50 mM Tris-HCl, 3% SDS, 1% β-mercaptoethanol, 20% glycerol, 0.7% bromophenol blue pH = 6.8) on 12.5% acrylamide gel. Electrophoresis was initiated at 80 V until samples had run through the stacking gel (approximately 30 minutes). The voltage was subsequently increased to 120 V, and electrophoresis was continued until the tracking dye had reached the bottom of the gel (approximately 2 hours). Proteins were stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich).

Genome sequencing and bioinformatics analysis

The DNA of phage JH2 was sent to Shanghai Biorefer Technology for sequencing. This company conducts direct sequencing on genomic DNA. Open reading frames have been predicted with the FrameD program (Toulouse Bioinfo INRA) with ATG, GTG, and TTG as possible start codons and a minimum size of 30 amino acids. Nucleotide and predicted amino acid sequences were compared in the databases of NCBI/GenBank; EMBL; PIR-Protein; SWISS-PROT; and PROPOSITE (Chibani-Chennoufi *et al*., 2004). Additional database searches have been conducted with BLAST (Altschul *et al*., 1997) and PSI-BLAST at the NCBI and FASTA (Lipman and Pearson, 1985; Chibani-Chennoufi *et al*., 2004). Prediction of the tRNAs gene was done with the tRNAscan-SE program (Lowe and Eddy, 1997).

Nucleotide sequence accession number

The sequence of the phage JH2 genome has been deposited in GenBank as accession number KF055347.

Results

Isolation and morphology of bacteriophages

A novel bacteriophage (named JH2) was isolated using O8: F4: ETEC strain as the host collected from a swine farm in Nanjing province, China. Phage JH2 formed medium-sized, clear, round plaques (about 2-2.5 mm in diameter) on an LB plate spread with the O8: F4: ETEC strain. Electron microscopy pictures show that phage JH2 has an icosahedral head, a neck and a contractile tail, with tail fibers (Fig. 1), which belongs to the *Myoviridae* family. Five images of the phage JH2 were measured. The mean values were as follows: the head was 75 nm length and 70 nm wide, while the tail was 100 nm length and 22 nm wide.

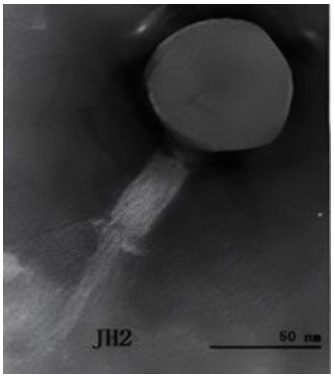

Fig. 1. Electron microscopic appearance of phage JH2.

One-step growth curve

After phages were infecting bacteria for 25-120 minutes, this solution was titrated to determine the number of phages. A one-step growth curve for phage JH2 showed a latent period of about 25 minutes, while the lysed period was 95 minutes. After 120 minutes, the number of phages reached the maximum and remained in a stable stage (Fig. 2).

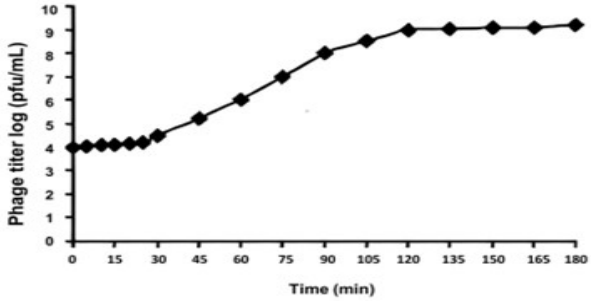

Fig. 2. One-step growth curve of phage JH2.

Host range

Bacteriophage JH2 specifically lysed pig clinical isolates of O8 *E. coli*, whereas neither chick or bovine *E. coli* strains, nor other O serogroup of *E. coli* strains, were sensitive. A high proportion (85 %, n = 32) of O8 ETEC strain isolates was sensitive to phage JH2.

Stability of bacteriophage JH2

Phage JH2 was found to be quite sensitive to heat (Fig. 3), as more than 55% of phage particles were killed after 30 minutes of incubation at 60°C, and only 10% of the phage particles were still alive after 120 minutes of incubation at 60°C. Less than 10% of phage particles survived after 30 minutes of incubation at 70°C. The bacteriophage JH2 was highly susceptible to acidity at pH 1 - 2 and susceptible in varying degrees to overnight exposure to pH 3 - 4 or 11. The bacteriophage was resistant to the range of pH 5 - 9 (Fig. 4).

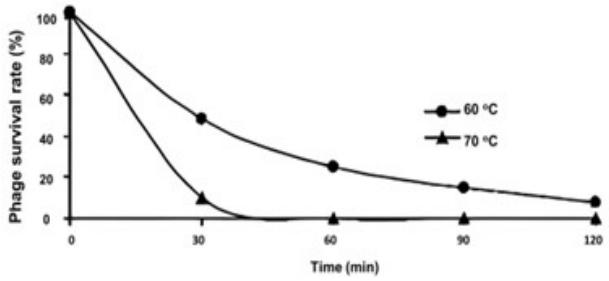

Fig 3. The curve of heat stability

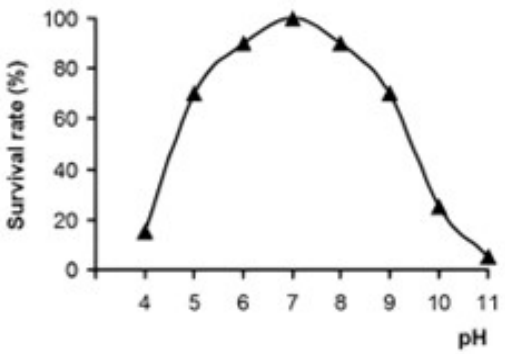

Fig. 4. The curve of pH stability

Proteins of JH2 phage particles

The phage was purified by a glycerol gradient procedure after purified particles were subjected to SDS-PAGE. The four bands 86, 53, 40, and 14-kDa JH2 (Fig. 5) were identified as being consistent with the products of ORFs 79, 83, 22, and 4, respectively.

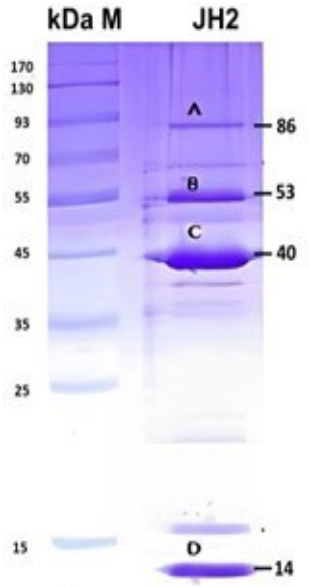

Fig. 5. SDS-polyacrylamide gel electrophoresis of bacteriophage JH2, Lane M, molecular weight markers.

The general feature of JH2 genome

The genome of phage JH2 (KF055347 GENBANK) was 87 kb, linear, double-stranded with a G + C content of 38.82% (Table 1). A total of 131 open reading frames (ORFs) were identified in the genome as probable protein-coding genes (Tables 3.1, 3.2, 3.3). One hundred twenty-six (126) ORFs could be assigned a putative function. Most ORFs of phage JH2 were transcribed on the negative strand, and thirty-eight ORFs were transcribed on the positive strand. Most of the ORFs contain an ATG start codon; two ORFs carried a TTG start codon. The longest stretch was 2367 bp (ORF 21). Most adjacent genes had either a short overlap or 0 to 37 nucleotides between them. All of the genes were read from the same DNA strand. One hundred and twenty-one ORFs were associated with some function and were assigned as putative genes with some specified function based on translated protein sequences and bioinformatic analysis (gene products, including DNA packaging proteins, morphogenetic proteins, lysis components, and proteins necessary for DNA recombination, modification, and replication). The genes are organized into functional clusters, of which one cluster is involved in the transition from host to phage machinery, another in the replication of phage DNA and a third in phage particle and DNA maturation and packaging.

Bioinformatics analysis of JH2 genome

The phage JH2 genome appeared to be modularly organized, consisting of gene clusters involved in DNA packaging, head and tail morphogenesis, lysogeny, replication, and regulation. Phage JH2 genome contained one gene ORF 36 with homology to HNH endonuclease. The JH2 morphogenesis module lies (Tables 3.1, 3.2, 3.3) gene for deoxynucleotide monophosphate kinase, ORF 54. The tail length of JH2 bacteriophage encoded by gene ORF 88. A major capsid protein was encoded by ORF 97. The head maturation protease was encoded by gene ORF 99, as well as the conserved proteins ORF 20, 83, 87, 92, and 95. While, the proteins of the fiber of JH2 were encoded by ORF 78 and 79.

Table 1. Characteristics of the genome-sequenced JH2 phage.

Characteristic	Number, function
dsDNA molecule	77178 bps
GC%	38.89
Nucleotides	77,044
Query Alignment	87.43
Sbjct Alignment	52
Depth	20738
OFRs	130
Putative function	124
Negative strand	85
Positive strand	39
ATG start codon	126
GTG start codon	3
ATG start codon	1
Function of ORFs	head, tail morphogenesis, lysogeny, replication and regulation

DNA replication module

Further downstream we find genes that are clearly involved in DNA replication and recombination. The JH2 genes belong to genes for DNA synthesis and nucleotide metabolic: JH2 encodes replicative primase/helicase ORF 52, deoxynucleotide monophosphate kinase ORF 54, DNA ligase (ORF 67), and DNA polymerase (ORF 57 and 59). It contains anaerobic NTP reductase as a small subunit of ORF 32, as well as dihydrofolate reductase ORF 74 and thymidylate synthase ORF 75, along with transcriptional regulator ORF 70.

tRNA

The JH2 genes contain a group of genes that belong to RNA synthesis and ribonucleotide metabolic. During the translation of phage rIIB and rIIA genes (ORFs 21 and 22), regulation occurs of messenger RNA synthesis. Its encoding includes the subunit of ribonucleotide diphosphate reductase β chain ORF 40, ribonucleotide triphosphate reductase α chain kinase ORF 42, oxygen-sensitive ribonucleotide-triphosphate reductase ORF 36, exodeoxyribonuclease ORF 48, putative nicotinate phosphoribosyl transferase ORF 25, and ribose-phosphate pyro phosphokinase ORF 26. The JH2 genome included 22 tRNA. The tRNA type and anticodons were characterized in the data (Table 2).

The tRNA included Pro, Glu, Met, Pseudo, Asp, Lys, Ile, Arg, Leu, Lys, Ala, Gly, Thr, Val, Leu, Arg, Gln, Leu, Gln, Pseudo, Phe, and Cys. It also included the codons in their tRNA: TGG, TTC, CAT, GTA, GTC, TTT, GAT, TCT, TAG, CTT, TGC, TCC, TGT, TAC, CAA, ACG, TTG, TAA, CTG, GTG, GAA, and GCA.

Unknown function

The precise delineation of the groups of genes is not possible because of the presence of many genes with no homology or genes that encode proteins of unknown function 5 ORF (13, 15, 51, 103, and 128). Five ORFs (33, 60, 63, 107, and 127) showed non-homology to sequencing in the databases. In several cases, protein homologies were with proteins of phages of the *Myoviridae*. Fig. 6 show that the JH2 genome is very similar (by 95%) to the sequence of the complete genome of *Staphylococcus* phage SA1 (GU16994.1 Genbank). Interestingly, this is the first discovery of an almost complete phage genome within the genome of another phage.

Discussion

Bacteriophages are ubiquitous throughout the world and extremely diverse (Swanson *et al*., 2012). Although recent research on bacteriophage is expanding, it is still limited (King *et al*., 2011). Isolate and characterize more bacteriophages will require the utilization of abundant bacteriophage resources. Due to their highly specific host recognition, phage has

Table 2. Phage JH2 was predicted to have tRNA sequence.

tRNA	tRNA coordinates	tRNA -Type	Anticodon	Covescore	Hmmscore	Strscore
1	74699-74623	Pro	TGG	68.25	39.21	29.04
2	74615-74538	Glu	TTC	65.23	30.59	34.64
3	74446-74370	Met	CAT	28.9	18.87	10.03
3	74145-74058	Pseudo	GTA	39.92	9.54	30.38
5	74051-73974	Asp	GTC	66.01	42.76	23.25
6	73516-73441	Lys	TTT	60.2	37.08	23.12
7	73357-73283	Ile	GAT	55.42	25.58	29.84
8	72946-72870	Arg	TCT	76.59	57.86	18.73
9	72286-72209	Leu	TAG	48.98	22.44	26.54
10	72201-72126	Lys	CTT	63.17	41.04	22.13
11	72119-72044	Ala	TGC	47.67	15.72	31.95
12	72037-71963	Gly	TCC	56.5	31.92	24.58
13	71955-71880	Thr	TGT	59.75	28.49	31.26
14	71784-71710	Val	TAC	51.84	24.58	27.26
15	71708-71631	Leu	CAA	46.15	19.28	26.87
16	71625-71550	Arg	ACG	64.96	51.43	13.53
17	70964	Gln	TTG	47.31	25.79	21.52
18	70886	Leu	TAA	57.88	33.67	24.21
19	70802	Gln	CTG	52.69	27.23	25.46
20	70695	Pseudo	GTG	38.8	4.88	33.92
21	70613	Phe	GAA	43.77	24.19	19.58
22	69688	Cys	GCA	48.87	12.73	36.14

Table 3.1. Summary of the gene content of functional modules present in the JH2 phage.

ORF	Strand	ORF start	ORF end	Size (aa)	Mol mass (kDa)	Indittation codon	GC %	Predicted -function	Gene product	E value	Identity %
1	-	28	261	77	8.7	ATG	38.46	Hypothetical protein	*Escherichia* phage EC6	1.00E-36	97.4
2	-	325	888	187	21.4	ATG	39.36	Hypothetical protein	*Salmonella* phage SPT	7.00E-98	92.47
3	-	981	1184	67	7.5	ATG	36.76	Hypothetical protein	*Escherichia* phage EC6	1.00E-29	98.48
4	-	1285	1689	134	15.1	ATG	39.75	Hypothetical protein	*Salmonella* phage SPT-1	4.00E-68	94.78
5	-	1775	2047	90	22.4	ATG	37.73	Hypothetical protein	*Escherichia* phage wV8	1.00E-44	95.56
6	-	2135	2467	110	12.7	ATG	36.34	Hypothetical protein	*Salmonella* phage SPT-1	8.00E-49	93.46
7	-	2461	2757	98	11.5	ATG	31.99	Hypothetical protein	*Salmonella* phage Felix01	4.00E-47	91.84
8	-	2850	3362	170	19.6	ATG	38.99	Hypothetical protein	*Staphylococcus* phage SA1	8.00E-87	91.18
9	-	3458	3715	85	9.4	ATG	36.82	Hypothetical protein	*Salmonella* phage Felix01	4.00E-24	69.05
10	-	3805	4185	126	14.2	ATG	38.06	Hypothetical protein	*Escherichia* phage EC6	2.00E-63	93.6
11	-	4274	4480	68	8.2	ATG	36.71	Hypothetical protein	*Salmonella* phage Felix01	2.00E-26	85.07
12	-	4988	5773	261	29.2	ATG	40.2	Hypothetical protein	*Salmonella* phageSPT-1	3.00E-144	94.25
13	-	5774	5974	66	7.4	ATG	41.79	Unknown	*Salmonella* phage Felix01	1.00E-30	96.97
14	-	5967	6200	77	8.8	ATG	38.46	Hypothetical protein	*Salmonella* phage Felix01	9.00E-21	66.23
15	-	6175	6501	108	12.6	ATG	37.31	Unknown	*Salmonella* phage Felix01	5.00E-54	95.37
16	-	6504	6773	89	10.5	ATG	38.52	Hypothetical protein	*Escherichia* phage EC6	1.00E-45	97.75
17	-	6933	7280	115	13.3	ATG	35.92	Hypothetical protein	*Escherichia* phage wV8	2.00E-60	98.26
18	-	7333	7797	154	16.3	ATG	40.43	Hypothetical protein	*Escherichia* phage wV8	2.00E-79	98.04
19	-	7809	8504	231	26.3	ATG	36.35	Hypothetical protein	*Staphylococcus* phage SA1	1.00E-132	99.13
20	-	8482	9030	182	20.8	ATG	35.34	Phage conserved protein	*Staphylococcus* phage SA1	2.00E-101	98.9
21	-	9131	10240	369	41.8	ATG	39.82	rIIB protein	*Escherichia* phage wV8	0	96.75
22	-	10320	12686	788	89.5	ATG	39.04	rIIA protein	*Staphylococcus* phage SA1	0	97.08
23	-	12715	12891	58	65.1	ATG	38.98	Hypothetical membrane protein	*Salmonella* phage Felix01	4.00E-25	96.55
24	-	12873	13208	111	12.8	ATG	35.71	Hypothetical protein	*Escherichia* phage	4.00E-58	99.1
25	-	13262	15043	593	66.3	ATG	42.2	Putative nictotinate, Phosphoribosyl transferase	*Escherichia* phage wV8	0	97.81
26	-	15090	15971	293	32.3	ATG	40.25	Putative ribose-phosphate, pyrophosphokinase	*Staphylococcus* phage SA1	8.00E-164	95.56
27	-	15987	16520	177	20.9	ATG	38.95	Hypothetical protein	*Staphylococcus* phage SA1 PE	10.00E-96	96.49
28	-	16572	16892	106	12.3	ATG	36.45	Hypothetical protein	*Escherichia* phage EC6	2.00E-52	99.06
29	-	16895	17161	88	9.2	ATG	43.45	Hypothetical protein	*Escherichia* phage EC6	5.00E-24	92.05
30	-	17177	17452	91	10.4	ATG	35.87	Hypothetical protein	*Escherichia* phage EC6	2.00E-42	92.31
31	-	17415	17900	161	18.7	ATG	40.95	Anaerobic NTP reductase, small subunit	*Escherichia* phage wV8	1.00E-90	98.76
32	-	17936	18331	131	15	ATG	38.38	Hypothetical protein	*Staphylococcus* phage SA1	6.00E-70	98.47
33	-	18531	18866	111	12.8	ATG	35.71	-	-	-	-
34	-	18929	19495	188	21.7	ATG	36.51	Hypothetical protein	*Salmonella* phage SBA	1.00E-101	96.2
35	-	19503	20369	288	32.8	ATG	40.14	Oxygen sensitive ribonucleoside triphosphate reductase	*Escherichia* phage EC6	1.00E-167	97.57
36	-	20613	21077	154	18.1	ATG	34.84	HNH homing endonuclease	*Salmonella* phage SBA	9.00E-80	93.51
37	-	21190	22389	399	44.9	ATG	38.25	Ribonucleotide reductase of class III (anaerobic) large subunit	*Salmonella* phage SPT-1	0	97.74
38	-	22438	22644	68	7.9	ATG	33.33	Hypothetical protein	*Escherichia* phage EC6	8.00E-28	97.06
39	-	22637	22879	80	9.1	ATG	39.92	Hypothetical protein	*Salmonella* phage SBA-1781	2.00E-39	97.5
40	-	22879	23952	357	41.3	ATG	36.69	Putative ribonucleoside diphosphate reductase beta chain	*Salmonella* phage Felix01	0	99.72
41	-	23949	24206	85	10.1	ATG	36.82	Hypothetical protein	*Staphylococcus* phage SA1	2.00E-41	95.29
42	-	24262	26496	744	85.1	ATG	40.72	Ribonucleoside triphosphate, reductase alpha chain	*Staphylococcus* phage SA1	0	100
43	-	26543	26782	79	9.3	ATG	38.33	Hypothetical protein	*Salmonella* phage Felix01	7.00E-39	96.2
44	-	26874	27197	107	12.7	ATG	37.96	Hypothetical protein	*Escherichia* phage EC6	9.00E-56	98.13
45	-	27178	27933	251	28.6	ATG	38.76	Hypothetical protein	*Salmonella* phage Felix01	2.00E-144	98.8
46	-	27926	28156	76	9	ATG	36.8	Hypothetical protein	*Escherichia* phage EC6	1.00E-30	95.83
47	-	28178	28675	165	19.3	ATG	35.34	Hypothetical protein	*Staphylococcus* phage SA1	5.00E-91	97.58
48	-	28665	29705	346	39.6	ATG	37.75	Putative exodeoxyribonuclease	*Staphylococcus* phage SA1	0	97.98
49	-	29768	30625	285	32	ATG	40.33	Hypothetical protein	*Escherichia* phage EC6	3.00E-164	100
50	-	30698	30847	49	5.6	ATG	37.33	Hypothetical protein	*Salmonella* phage SPT-1	3.00E-19	100
51	-	30844	31125	93	11.3	ATG	33.69	Unknown	*Salmonella* phage Felix01	2.00E-43	91.4
52	-	31100	33085	661	74.8	ATG	41.94	Putative phage DNA primase/helicase	*Escherichia* phage wV8	0	99.7
53	-	33078	33272	64	7.6	ATG	35.9	Hypothetical protein	*Salmonella* phage Felix01	8.00E-27	100
54	-	33287	34045	252	29.1	ATG	37.15	Putative deoxynucleotide, monophosphate kinase	*Staphylococcus* phage SA1	2.00E-136	95.55
55	-	34092	34904	270	30	ATG	39.61	Hypothetical protein	*Staphylococcus* phage SA1	4.00E-135	87.78

Table 3.2. Summary of the gene content of functional modules present in the JH2 phage.

ORF	Strand	ORF start	ORF end	Size (aa)	Mol mass (kDa)	Inditation codon	GC %	Predicted -function	Gene product	E value	Identity %
56	-	34906	35328	140	16.5	ATG	37.83	Hypothetical protein	*Staphylococcus* phage SA1	4.00E-76	100
57	+	35586	37769	727	83.9	ATG	40.44	DNA polymerase	*Salmonella* phage SBA-1781	0	98.9
58	+	37837	38319	160	18.6	ATG	41.2	Hypothetical protein	*Salmonella* phage SBA-1781	1.00E-86	96.88
59	+	38533	39087	184	20.4	TTG	41.08	DNA polymerase	*Salmonella* phage SBA-1781	6.00E-101	97.28
60	+	39149	39367	72	8	ATG	37.44	-	-	-	-
61	+	39364	39573	69	8.07	ATG	38.1	Hypothetical protein	*Salmonella* phage Felix01	3.00E-32	100
62	+	39570	39782	70	7.9	ATG	39.91	Hypothetical protein	*Escherichia* phage EC6	1.00E-31	94.29
63	+	39800	39955	51	6.3	ATG	35.9	-	-	-	-
64	+	39969	40205	78	8.7	ATG	38.82	Hypothetical protein	*Escherichia* phage wV8	6.00E-38	96.15
65	+	40216	40422	68	7.8	ATG	41.55	Hypothetical protein	*Escherichia* phage wV8	5.00E-31	98.53
66	+	40419	40586	55	6.4	ATG	33.33	Hypothetical protein	*Escherichia* phage wV8	7.00E-19	92
67	+	40736	41875	379	44.1	ATG	40.7	Putative DNA ligase	*Staphylococcus* phage SA1	0	98.42
68	+	42152	42535	127	14.2	ATG	37.51	Hypothetical protein	*Salmonella* phage Felix01	9.00E-69	99.21
69	+	42537	42749	70	8.1	ATG	35.68	Hypothetical protein	*Escherichia* phage wV8	3.00E-32	100
70	+	42742	43041	99	11.5	ATG	37.33	Putative Transcriptional regulator	*Escherichia* phage EC6	9.00E-52	100
71	+	43043	43402	119	13.7	ATG	38.89	Hypothetical protein	*Staphylococcus* phage SA1	1.00E-63	99.16
72	+	43416	43931	171	19.4	ATG	36.24	Hypothetical protein	*Staphylococcus* phage SA1	1.00E-89	95.91
73	+	43932	44192	86	9.7	ATG	37.93	Hypothetical protein	*Salmonella* phage Felix01	7.00E-44	98.84
74	+	44189	44734	181	20.4	ATG	36.81	Dihydrofolate reductase	*Escherichia* phage EC6	8.00E-98	96.69
75	+	44736	45635	299	34.2	ATG	39.78	Thymidylate synthase	*Escherichia* phage wV8	2.00E-175	98.33
76	-	45672	46043	123	13.7	ATG	36.56	Hypothetical protein	*Escherichia* phage wV8	8.00E-63	97.56
77	-	46040	46237	65	7.6	ATG	34.85	Hypothetical protein	*Escherichia* phage wV8	3.00E-27	98.41
78	-	46317	48662	781	83.6	ATG	40.2	Putative tail fiber protein	*Salmonella* phage Felix01	0	76.82
79	-	48709	49878	389	40.9	ATG	42.22	Putative tail fiber protein	*Staphylococcus* phage SA1	5.00E-175	83.8
80	-	49881	50183	100	11	ATG	38.28	Hypothetical protein	*Salmonella* phage Felix01	7.00E-50	97
81	-	50183	51040	285	31.7	ATG	35.55	Hypothetical protein	*Staphylococcus* phage SA1	4.00E-158	95.44
82	-	51043	52512	489	53.2	ATG	38.23	Hypothetical protein	*Salmonella* phage SBA-1781	0	96.73
83	-	52512	52931	139	15.6	ATG	34.29	Phage conserved protein	*Salmonella* phage Felix01	2.00E-75	99.28
84	-	52931	53554	207	23.1	ATG	38.78	Hypothetical protein	*Escherichia* phage EC6	7.00E-120	99.52
85	-	53554	54531	325	37.1	ATG	35.07	Hypothetical protein	*Escherichia* phage EC6	0	99.08
86	-	54531	54872	113	13.2	ATG	35.96	Hypothetical protein	*Escherichia* phage wV8	1.00E-59	99.12
87	-	54872	55669	265	28.7	ATG	38.97	phage conserved protein	*Staphylococcus* phage SA1	2.00E-152	100
88	-	55669	57912	747	81.3	ATG	41	Tail length tape-measure protein	*Salmonella* phage SPT-1	0	92.1
89	-	57912	58151	79	9.1	ATG	37.08	Hypothetical protein	*Salmonella* phage Felix01	6.00E-38	98.73
90	-	58154	58552	132	14.8	ATG	34.84	Hypothetical protein	*Salmonella* phage Felix01	5.00E-69	96.97
91	-	58626	59072	148	16.2	ATG	41.61	Hypothetical protein	*Escherichia* phage wV8	2.00E-81	99.32
92	-	59088	60440	450	48.8	ATG	40.95	Phage conserved structural protein	*Salmonella* phage Felix01	0	96.22
93	-	60441	61040	199	22.3	ATG	37.33	Hypothetical protein	*Salmonella* phage Felix01	7.00E-112	100
94	-	61015	61416	133	15.6	ATG	36.32	Hypothetical protein	*Salmonella* phage Felix01	5.00E-73	100
95	-	61413	61895	160	17.7	ATG	38.3	Phage conserved protein	*Salmonella* phage Felix01	2.00E-87	99.38
96	-	61895	62344	149	16.9	ATG	40.67	Hypothetical protein	*Salmonella* phage Felix01	3.00E-81	98.66
97	-	62365	63471	368	41.4	ATG	46.16	Major capsid protein	*Staphylococcus* phage SA1	0	98.64
98	-	63505	63882	125	13.6	ATG	42.59	Hypothetical protein	*Salmonella* phage Felix01	1.00E-62	93.6
99	-	63894	65240	448	48.3	ATG	38.46	Putative head maturation protease	*Salmonella* phage Felix01	0	99.78
100	-	65252	65584	110	11.7	ATG	39.94	Hypothetical protein	*Salmonella* phage Felix01	1.00E-56	100
101	-	65584	66084	166	18.2	ATG	35.33	Hypothetical protein	*Salmonella* phage SPT-1	5.00E-91	99.4
102	-	66084	67550	488	55.4	ATG	38.24	Hypothetical protein	*Salmonella* phage SPT-1	0	99.59
103	-	67567	69168	533	60.1	ATG	41.45	Unknown	*Salmonella* phage Felix01	0	99.81
104	-	69190	69390	66	7.4	ATG	35.82	Hypothetical protein	*Salmonella* phage Felix01	8.00E-28	98.48
105	-	69787	70521	244	25.5	ATG	39.86	Hypothetical protein	*Escherichia* phage EC6	5.00E-117	96.72
106	-	70973	71530	185	21.9	ATG	37.81	Hypothetical protein	*Salmonella* phage SPT-1	4.00E-101	96.22
107	+	71694	71813	39	4.5			-	-	-	-
108	+	74495	74620	41	4.7	ATG	36.51	Hypothetical protein	*Salmonella* phage SBA-1781	6.00E-15	97.56
109	-	74865	76280	471	53.1	ATG	41.31	Hypothetical protein	*Staphylococcus* phage SA1	0	98.3
110	-	76362	76733	123	13.2	ATG	42.74	Hypothetical protein	*Staphylococcus* phage SA1	2.00E-63	99.19
111	+	76983	77144	53	6.4	ATG	34.57	Hypothetical protein	*Escherichia* phage EC6 PE	7.00E-23	100
112	+	77353	77943	196	22.1	ATG	35.03	Hypothetical protein	*Escherichia* phage EC6	3.00E-108	98.47

Table 3.3. Summary of the gene content of functional modules present in the JH2 phage.

ORF	Strand	ORF start	ORF end	Size (aa)	Mol mass (kDa)	Indittation codon	GC %	Predicted -function	Gene product	E value	Identity %
113	+	77991	78362	123	14.2	ATG	37.37	Hypothetical protein	*Escherichia* phage EC6	9.00E-64	94.31
114	+	78359	78991	210	23	ATG	42.18	Hypothetical protein	*Escherichia* phage EC6	2.00E-110	92.82
115	+	78991	79455	154	17.2	ATG	40.86	Hypothetical protein	*Salmonella* phage SPT-1	9.00E-86	100
116	+	79506	79904	132	15.1	ATG	37.84	Hypothetical protein	*Salmonella* phage Felix01	7.00E-60	81.82
117	+	79897	80295	132	15	ATG	43.11	Hypothetical protein	*Escherichia* phage wV8	5.00E-53	86.36
118	+	80295	80588	97	11.4	ATG	38.44	Hypothetical protein	*Escherichia* phage EC6	1.00E-49	100
119	+	80581	80925	114	13.7	ATG	33.62	Hypothetical protein	*Escherichia* phage wV8	7.00E-58	96.49
120	+	80925	81506	193	21.8	ATG	40.38	Hypothetical protein	*Salmonella* phage SPT-1	8.00E-109	98.45
121	+	81579	82124	181	20.6	ATG	38.1	Hypothetical protein	*Salmonella* phage Felix01	4.00E-93	90.06
122	+	82121	82339	72	8.7	ATG	36.99	Hypothetical protein	*Salmonella* phage Felix01	2.00E-32	95.83
123	+	82336	82839	167	19.5	ATG	37.1	Hypothetical protein	*Staphylococcus* phage SA1	5.00E-92	97.01
124	+	82916	83140	74	8.6	ATG	36.44	Hypothetical protein	*Escherichia* phage EC6	1.00E-25	95.95
125	+	83194	83658	154	17.7	ATG	33.76	Hypothetical protein	*Salmonella* phage Felix01	3.00E-79	93.51
126	+	83672	84220	182	21	ATG	38.07	Hypothetical protein	*Staphylococcus* phage SA1	10.00E-103	99.45
127	+	84850	84984	44	4.9	ATG	34.07	-	-	-	-
128	-	85916	86164	82	9.8	ATG	37.75	Unknown	*Salmonella* phage Felix01	3.00E-39	95.12
129	-	86230	86493	87	9.9	ATG	35.23	Hypothetical protein	*Salmonella* phage SBA-1781	1.00E-39	93.1
130	-	86559	87089	176	20	ATG	36.91	Hypothetical protein	*Salmonella* phage Felix01	6.00E-89	90.91
131	-	87310	87651	113	13.1	ATG	39.18	Hypothetical protein	*Salmonella* phage SPT-1	3.00E-58	93.81

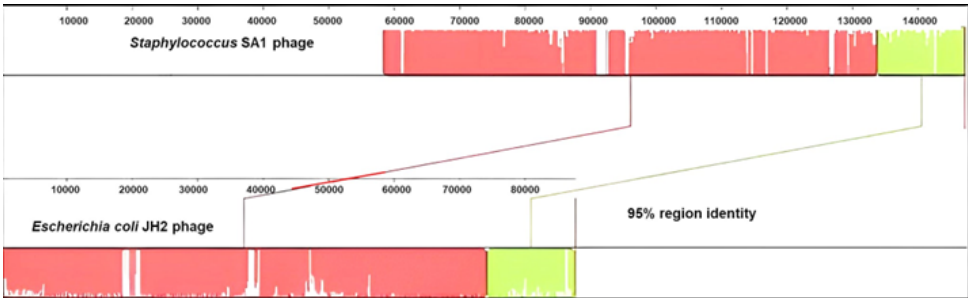

Fig. 6. Comparison with BLAST of JH2 and *Staphylococcus* SA1 genomes (*Staphylococcus* phage SA1 contains an almost complete phage genome of JH2 phage).

great potential as therapeutic agents in the treatment of certain human, animal, and plant bacterial infections (Jamalludeen *et al*., 2009b; Balogh *et al*., 2010; Abedon *et al*., 2011). In this study, we isolated a novel bacteriophage named JH2 from fecal samples. Morphological characteristics were seen under an electron microscope. In the last 45 years, 96% of phages of the *Siphoviridae*, *Myoviridae*, and *Podoviridae* families were investigated (Kumari *et al*., 2009). Based on morphological features and contractile tails, the phage JH2 against O8 *E. coli* in our study were members of the *Myoviridae* family. This family consists of six genes and is characterized by having an icosahedral or elongated head and a contractile tail that is more or less rigid, long, and relatively thick (Ackermann, 2011).

Phage JH2 was tested for its ability to lyse host ranges on the O8 ETEC, the predominant porcine PWD *E. coli* strains. Most of the O8 *E. coli* strains were lysed by the phage JH2. These variations might be caused by the function of the phage and the physiological state of the host (Flayhan *et al*., 2012). Phage JH2 was susceptible in varying degrees to overnight exposure to pH 3-4. The phage was often quite sensitive to protein denaturation in an acidic environment, which may result in a loss of viability of the phage. The ability to survive well over the pH range between 5 and 9 is a common feature of most phages. The pH in the stomach of weaned pigs may be as low as 1-2 before a meal and may rise quickly to 4-5 after the meal, depending on the diet and the feeding regime (Snoeck *et al*., 2004). The phage JH2 was likely to undergo a marked reduction in titer following oral administration to pigs unless steps were taken to reduce their exposure to low pH in the stomach and upper small intestine.

The JH2 genome appears to be linear, double-stranded permuted, and terminally redundant. In addition, the phylogeny of the *Caudovirales* large-subunit terminase proteins is correlated with the virus packaging strategy (Casjens and Gilcrease, 2009). Phage JH2 is unique since it is the first member of the *Myoviridae* which apparently possesses a *Staphylococcus* phage SA1 (GU16994.1 Genbank) transcriptional system. The intermingling of head and tail genes is probably an ancestral character because it was also observed in *E. coli* phage JS98 (Chibani-Chennoufi *et al*., 2004). The fiber adhesion domains are located in different genes in T-even phages. In phage T4, the adhesion is located in the C-terminal domain of gp37, while in the other T-even phages, the adhesion is a separate protein, gp38 (Te´tart *et al*., 1998; Chibani-Chennoufi *et al*., 2004).

The phage genome usually encodes few or no tRNA genes because they use the host protein synthesis machinery. Bacteriophage JH2 has 22 tRNA genes. The presence of an intron in the putative tRNA gene is to our knowledge the first such example in a phage genome (Jamalludeen *et al*., 2008). Group 1 introns that are self-splicing are found in a number of tRNA genes of alphaproteobacteria and cyanobacteria (Bonocora and Shub, 2001), and similar introns have been found in protein-encoding genes but not tRNA genes of phages (Belle *et al*., 2002). The significance of the intron in the tRNA gene is unknown. In the genome of phage JH2 no genes with homology to bacterial toxins were present, which would make the phage JH2 a candidate for phage therapy. This is an important finding since therapeutic and decontaminating phages should not be capable of integrating into the host chromosome to form lysogens.

Staphylococcus phage SA1 is dsDNA, the G+C content of 45.8%. No RNA belongs to the order *Caudovirales*, *Myoviridae* family (Lee *et al*., 2009). Since the sequence of *Staphylococcus* phage SA1 is related to that of the terminases from mycovirus, which are known to package DNA by a headful mechanism, we assumed that phage JH2 behaves similarly (Lee *et al*., 2009). The sequence of JH2 identifies 95% with the sequence of *Staphylococcus* phage SA1 (GenBank accession number, GU169904). However, the SA1 genome comprises 147,303 bp, which is 1.7 times larger than the JH2 genome. Also, *Staphylococcus* phage SA1 contains an almost complete phage genome of JH2 phage. The high identity of the two genomes obviously points to an evolutionary link between JH2 and SA1. However, the precise nature of this link remains unclear given that they were isolated in geographically distant regions: JH2 was isolated from China, while SA1 was identified in South Korea. In addition, JH2 and SA1 were isolated from different host species: Escherichia and *Staphylococcus*, respectively.

The presence of a sequence identical to the nearly complete genome of JH2 in the genome of SA1 suggests the existence of a common host and a common habitat for the two phages in the recent past. It seems

likely that this common host is a bacterium of the genus *Staphylococcus*. The identical phage sequences were discovered in habitats as geographically and ecologically distant as Antarctica and China (Swanson *et al*., 2012). However, numerous studies have reported the global distribution of at least some bacteriophages (Thurber, 2009).

Conclusion

A new bacteriophage, JH2, specifically infecting pig O8 ETEC strains was isolated and characterized in this study. Morphological analysis indicated that JH2 is a member of the family *Myoviridae*. Its complete genome sequence obtained here is the first reported for O8 ETEC phage. Complete genome sequence of bacteriophage JH2 showed great similarity to the genome sequence of *Staphylococcus* phage SA1.

Acknowledgments

This study was supported by a grant from the Program for New Century Excellence in the University of the Ministry of Education of China (NCET-110671) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Conflict of interest

The authors declare that they have no conflict of interest.

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