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Characterization and genomic analysis of a novel *E. coli* lytic phage with extended lytic activity against *S.* Enteridis and *S.* Typhimurium

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Abstract

In order to effectively use of phages as antimicrobial agents for controlling multidrug-resistant *E. coli*, it is important to understand phage biology. The isolation and research of novel bacteriophages are urgently needed for food safety and animal health. Phage is considered as a novel alternative antibacterial agents and a new way of prevent, control and treat pathogens. In this paper, we isolated and characterized a novel lytic phage from chicken samples, named vB_EscP_vE20 (in brief vE20). We identified vE20 as belonging to the *Podoviridae* family through morphological and phylogenetic analysis. It had lytic activities against 24 out of 51 different serotypes of clinical *E. coli* strains, interestingly it also can lyse *Salmonella* species, such as *Salmonella* Enteritidis (*S.* Enteritidis) and *Samonella* Typhimurium (*S.* Typhimurium). One step growth curve showed that the latent period and lysis period was 10 min and 60 min, respectively. The burst size of vE20 was about 60 PFU/cell. The phage vE20 survived in a wide range of temperatures (30–60 °C) and pH (3–11). The whole genome size of phage vE20 is 77,938 bps, which is double stranded DNA. There are 121 Open reading frame (ORFs) in total, and the GC-content is 42.17%, without virulence-associated, antibiotic and lysogeny related genes. Phage vE20 has high bactericidal activity in killing *E. coli* EXG20-1 in cultures containing 4×10^9 cfu/mL in several MOIs culture. The results revealed the promising potential of phage vE20 as attractive candidates for the control of *E. coli* infections.

Keywords *E. coli*, Lytic phage, Characterization, Genome analysis, *S.* Enteritidis, *S.* Typhimurium

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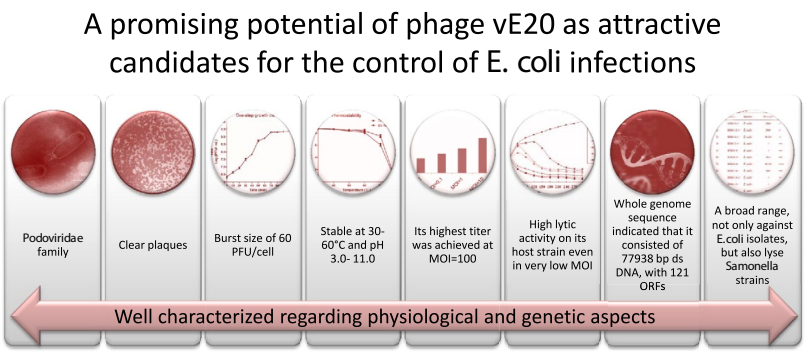
Graphical Abstract



Introduction

Escherichia coli the most commonly found Gram-negative species in human and animal gut flora, including benign normal flora and pathogenic bacteria (Kyle et al. 2012; Zhou et al. 2015), such as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC) (Gomes et al. 2016). EPEC and EHEC infections can cause severe gastrointestinal ailments such as hemorrhagic enteritis, bloody diarrhea, and extraintestinal diseases (Croxen et al. 2013). Recently, there has been a rise in food poisoning incidents caused by *E. coli*, making it necessary to prioritize food safety, particularly with regards to *E. coli* contamination (Lee et al. 2020; Malik et al. 2021). Although antibiotics are generally used to treat or control *E. coli* infections (Matsuzaki et al. 2005), the high prevalence of multidrug resistance (MDR) makes it essential to develop more effective treatment or control strategies (Caflisch et al. 2019; Rosner and Clark 2021; Pan et al. 2020). The emergence of multi-drug resistant bacterial strains is increasing. Therefore, developing new treatment/control strategies as alternatives to traditional antibiotics is crucial.

Phage, known as the natural predators of bacteria, may be the most numerous biological entities on earth (Brussow 2005). It causes specific infection of bacteria, so can achieve the effect of sterilization (Merabishvili et al. 2012). Phages have several advantages over antibiotics, including a strong antibacterial effect, ease of isolation and preparation, high efficiency in infection without affecting normal flora, and few side effects (Styles et al. 2022; Gordillo and Barr 2019; Kortright et al. 2019). Thus, the food and medical industries are gradually applied the lytic phages (Jamal et al. 2019). In the early 2006, the US Food and Drug Administration (FDA) approved the use of phages as a new type of food preservative for fresh vegetables and meat products (Safwat et al. 2018). Many studies showed that phages can be used successfully used to treat pathogenic bacteria in the food chain, including zoonotic pathogens that colonize the intestines of farm animals. Moreover, it is possible to develop alternative treatment methods either for food safety, veterinary or clinical use through discovering new phages effective against pathogenic bacteria. Therefore, the isolation and research of novel bacteriophages are urgently needed for food safety and animal health.

In this work, we isolated a novel lytic *E. coli* phage from chicken samples and analyzed its biological characteristics and genomic information. Phage vE20 has a wide host range, rapid cleavage cycle, high burst volume, wide temperature and PH tolerance, no virulence related genes, antibiotics and lysogen-related genes. Furthermore, the isolation and research of phage vE20 provides more basis of reference value to fight against *E. coli.*

Materials and methods

Host of phage vE20

The host of phage vE20 was an enteropathogenic *Escherichia coli* strain EXG20-1, which was isolated from chicken meat product in a nearby market and identified by standard microbiological methods according to the National Standard of China (GB/T 4789.4–2016).

Culture media and grow conditions

All strains grew in Luria Bertani broth at 37 °C (LB, Qingdao Hope BioTechnology Co. Ltd., Qingdao, China) and agar plate overnight, under aerobic conditions.

Bacteriophage isolation and purification

The sewage samples from chicken were centrifuged at 12,000 rpm/min for 30 min at 4 °C. Then, the supernatant

were incubated with *E. coli* EXG20-1 on shaking at 37 °C overnight (90 rpm). Afterwards, the mixture was centrifuged at 12,000 rpm/min for 10 min. The supernatant was subjected to 0.22 μm membrane filtration, using the double-agar layered method as previously described to isolate phage (Bao et al. 2011). Each phage was repeated this process 8–10 times to obtain pure phage strains.

Preparing phage particles and electron microscopy

The supernatant added polyethylene glycol (Amresco, Solon, USA, 8000) and NaCl. The concentrations reached 10% (w/v) and 0.5 M (mol/L) finally. Collecting the phage particles by centrifugation at 11, 000×*g* for 10 min at 4 °C and dissolved in SM buffer (5.8 g/L of NaCl, 2.0 g/L of $MgSO_4$, 50 mL/L of 1 M Tris, pH 7.5, 5 mL/L of pre-sterilized 2% gelatin). Phage particles were processed by negative stain with 2% phosphotungstic acid (PTA, Sigma-Aldrich, St. Louis, USA) and examined by an H-7650 electron microscopy (Hitachi High- Technologies Co., Tokyo, Japan).

Multiplicity of Infection (MOI)

The optimal MOI for separating bacteriophages was determined using the previously method (Xi et al. 2019; Zhai et al. 2019). In short, phages were added to the host bacterial solution (1.0×10^8 CFU/mL) to achieve the multiplicity of infection (MOI) of 0.01, 0.1, 1, and 10, and incubating at 37 °C and 180 r/min for 5 h with shaking. The phage titer in the lysate was determined by the double-layer plate method, and the multiplicity of infection with the highest titer was the optimal MOI of the phage.

Phage lytic ability

The overnight cultured *Escherichia coli* EXG20-1 was transferred to 5 mL of LB liquid medium at a ratio of 1:100, and placed in a shaking incubator (37 °C, 180 r/min) and reached a concentration of 4×10^9 CFU/mL. Each 5 mL of the diluted bacterial culture was transferred into 10-mL tubes. For each tube, a concentration of phage stock was added to achieve an initial MOI of 0, 0.00001, 0.0001, 0.001, 0.01, 0.1 or 1. These tubes were incubated without shaking at 37 °C and the OD_{600} value of each tube was measured at 30-min intervals for a 5-h period using an Ultrospec™ cell density meter (GE Healthcare, San Diego, Calif., USA). The experiment was performed 3 times.

One-step growth curves

For the one-step growth curve, the method described earlier was used, but minor modifications were made (Bao et al. 2011). Briefly, adding suspension to log-phase culture at the optimal MOI and incubating at 37 °C for 15 min, then, centrifuging the mixture at 13,000×*g* for 1 min at 4 °C and the supernatant was discarded. The pellets containing (partially) infected cells were resuspended in 10 mL of pre-warmed TSB (1×10^7 CFU/mL) and were incubated again at 37 °C without shaking. Samples were taken at 10-min intervals (up to 2 h), then immediately diluted, and plated for the next study.

Thermal and pH stability

In the thermo-stability assay, the phage isolations were incubated at 30, 40, 50, 60, and 70 °C, and the aliquots were collected after 30 min, and 60 min to be titered by the double-layer agar method. For the pH stability assay, 100 μL of the phage suspension was added to tryptone water (900 μL) with pH 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 9.0, 10.0, 11.0 and 12.0, respectively, incubated for 2 h at a 37 °C water bat. Then, they were titered by the double-layer agar plate method.

Phage lytic ability

We studied the lytic activity of phage vE20 on its host strain EPEC EXG20-1 under five different MOIs. Diluting overnight bacterial culture with LB to a concentration of 1×10^9 CFU/mL, then, transferred 5 mL of diluted bacterial culture into 10-mL tubes. One of the tubes serves as a control. Adding 10-serial diluted different titer phage stocks (1×10^9 PFU/ml ~) to each of the other tubes to obtain an initial MOI of 0, 0.00001, 0.0001, 0.001, 0.01, 0.1 or 1. Incubating these test tubes at 37° C, and measuring the OD_{600} of each tube at 30-min intervals within 5-h using an Ultrospec™ cell density meter (GE Healthcare, San Diego, California, USA). The experiment was conducted three times.

Phage DNA isolation and genome sequencing

For the extraction and sequencing of phage genome DNA, it referred to the previous method with slight modifications (Bao et al. 2019). Take an appropriate amount of purified and filtered phage solution, add DNase I and RNase A (Takara, Shanghai, China) to 1 μg/mL, incubate at 37 °C for 30 min, add 50 μL of 0.5 mol/L EDTA and treat at 65 °C for 10 min to terminate the endonuclease reaction. Protease (50 μg/mL, Sigma, USA) and sodium dodecyl sulfate (SDS, 0.5% of final concentration, Beijing Dingguo Changsheng Biotechnology Co. LTD, Beijing, China) were added, and the mixture was incubated overnight at 56 °C. Extraction with phenol–chloroform-isoamyl alcohol (25:24:1, volume ratio) was repeated twice, the pellet after centrifugation was washed with 75% ethanol, and the phage genome was dissolved in distilled water with 50 μg/mL RNase A.

The whole genome sequencing was entrusted to Wuhan Bena Technology Service Co, Ltd., and the sequencing data were assembled using SeqMan II

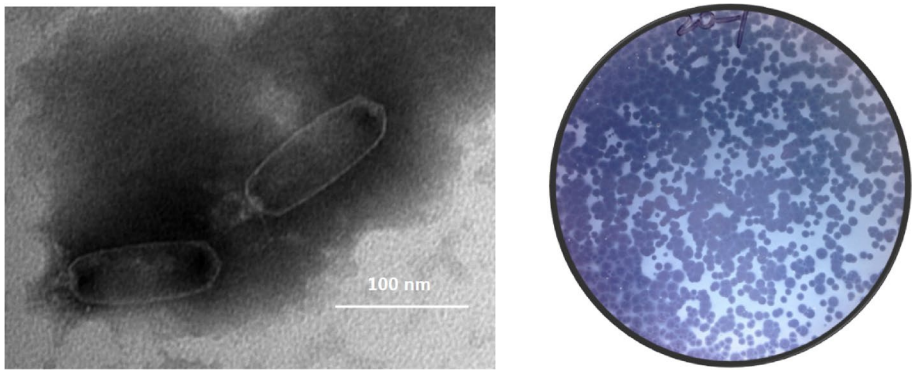


Fig. 1 The plaques and morphology of phage vE20

sequence analysis software (DNASTAR Inc., USA). The protein alignment search tool BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to predict the function of protein sequences based on homology to known protein sequences. The whole genome sequence of phage vE20 was uploaded to the GenBank database with the sequence number OP293233.

Phage genome alignment analysis and phylogenetic tree construction

The phage genomes were aligned using the BLASTn online tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the enterococcal phage genomes with similar similarity were downloaded, and the genomes were aligned and analyzed based on the nucleic acid level by MAUVE 2.3.1. Similarity analysis was also performed on the amino acid sequences encoded by lyases and capsid proteins, and an evolutionary tree was constructed based on the amino acid sequences by the software MEGA 7.0.

Results

Isolation of phage vE20 and its morphology

Phage vB_EscP_vE20 (simple as vE20) was isolated from chicken stool sewage by using the double-layer plate method. *E. coli* EXG20-1, serotype O128, was the host strain of phage vE20, and the plaques were purified 8–10 time. Phage vE20 was capable to produce clear plaques on its host's lawns. Electron microscopy revealed that phage vE20 belongs to the *Podoviridae* family. The dimensions of vE20 were about 125.4 nm for the head and 15.7 nm for the short tail (Fig. 1).

Multiplicity of Infection (MOI) and one-step growth curve

We found that highest titer of vE20 could reach 1.03×10^{10} PFU/mL under the MOI was equal to 100 (Table 1). The one-step growth curve of phage vE20 was determined under the optimal MOI. The latent period was approximately 10 min, outbreak period is

Table 1 Determination of optional multiplicity of infection of phage vE20

No	Bacteria (CFU/mL)	Number of phage (PFU/mL)	MOI	Phage titer (PFU/mL)
1	5.4×10^{7}	5×10^{5}	0.01	2.95×10^{9}
2	5.4×10^{7}	5×10^{6}	0.1	3.95×10^{9}
3	5.4×10^{7}	5×10^{7}	1	5×10^{9}
4	5.4×10^{7}	5×10^{8}	10	7.1×10^{9}
5	5.4×10^{7}	5×10^{9}	100	1.03×10^{10}

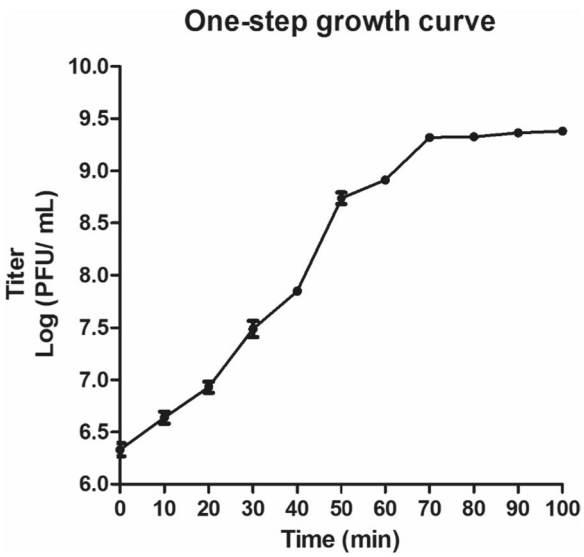


Fig. 2 One-step growth curve of phage vE20

about 60 min and the burst size was about 60 PFU/cell (Fig. 2).

Stability of vE20

Analysis of the stability showed that phage vE20 was relatively stable under the action of 30–60 °C for 30 min and 60 min, and the phage was inactivated under the action of 80 °C for 30 min (Fig. 3). Furthermore, phage vE20 was found that it could be stable among pH 3.0 and 11.0 by plaque counting (Fig. 3).

Host range

Phage vE20 had activity against a wide range of *E. coli* and some *Salmonella* isolates (*S.* Enteritidis ATCC13076 and *S.* Typhimurium ATCC13311). Moreover, the vE20 had a lytic effect on 47.05% (24 out of 51 isolates) of *E. coli* clinical isolation (Table 2).

Phage infection

The lytic activity of vE20 against its natural host EXG20-1 in liquid culture was investigated at 7 different MOI (Fig. 4). The phage-free culture (MOI = 0) grew at a steady

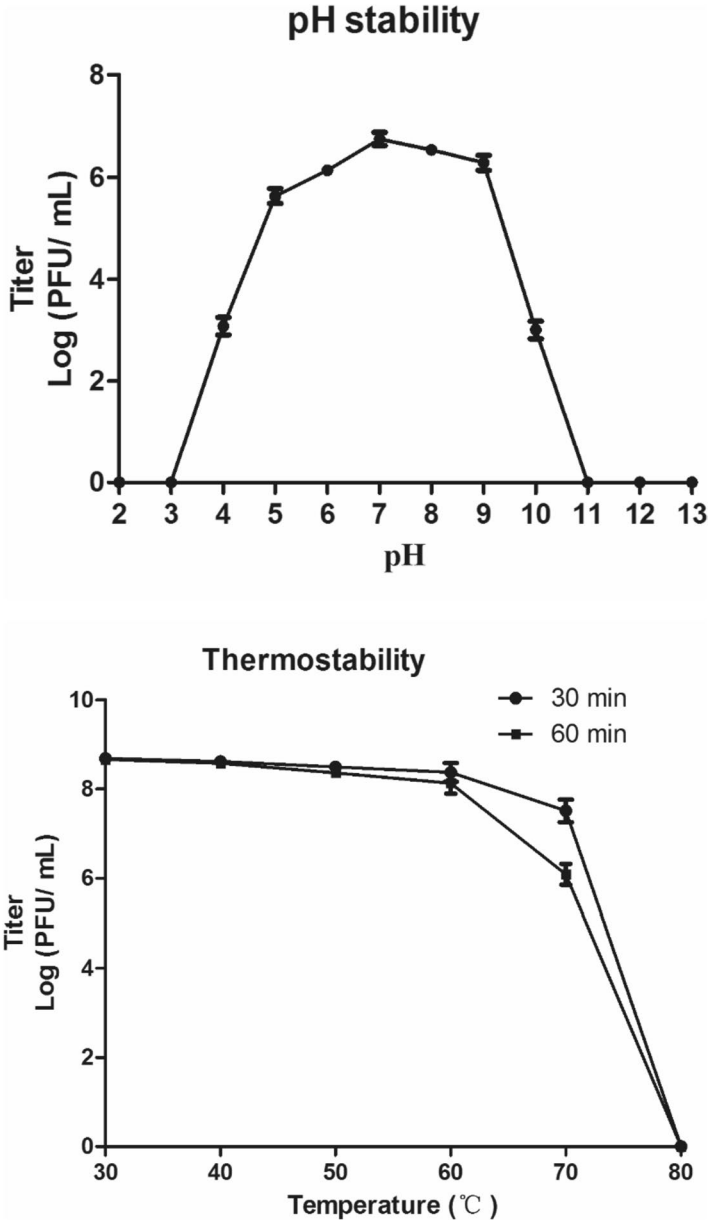


Fig. 3 Determination of stability of phage vE20

Table 2 Host range of phage vE20

No	Strains	Species	Serotype	Phage vE20 susceptibility
1	EXG2-2–1	*E. coli*	O44	-
2	EXG15-2–1	*E. coli*	O44	-
3	EXG3-1–1	*E. coli*	O128	+
4	EXG3-2–1	*E. coli*		+ +
5	EXG26-1	*E. coli*		+ +
6	EXG26-2	*E. coli*	O1	+ +
7	EXG20-1	*E. coli*	O128	+ + +
8	EXG21-1	*E. coli*	O8	+ +
9	EXG15-1–1	*E. coli*		+
10	EXG11-2	*E. coli*		+
11	EXG20-2	*E. coli*	O128	+ + +
12	EXG2-1–1	*E. coli*	O6	-
13	EXG28-3	*E. coli*		-
14	EXG8-2	*E. coli*	O78	-
15	EXG21-3	*E. coli*	O20	+ +
16	EXG21-2	*E. coli*	O20	+ + +
17	EXG5-2	*E. coli*	O18	+
18	EXG4-1	*E. coli*	O1	-
19	EXG6-1	*E. coli*	O1	-
20	EXG13	*E. coli*		-
21	K99	*E. coli*	O18	-
22	rb4	*E. coli*		+
23	rc5	*E. coli*	O128	+
24	rb3	*E. coli*	O1	+ +
25	rb2	*E. coli*	O20	-
26	rc2	*E. coli*	O86	+ + +
27	rc3	*E. coli*	O1	-
28	rc1	*E. coli*	O86	+
29	rh1	*E. coli*	O78	+ +
30	rc4	*E. coli*	O1	-
31	rh3	*E. coli*	O1	-
32	rp2	*E. coli*	O146	-
33	rp3	*E. coli*	O18	-
34	rb1	*E. coli*	O128	+ + +
35	rp4	*E. coli*	O128	+
36	rp5	*E. coli*		-
37	rh5	*E. coli*		-
38	rp22	*E. coli*	O1	-
39	rh2	*E. coli*		-
40	rh4	*E. coli*		-
41	rp6	*E. coli*		-
42	CVCC 197	*E. coli*	O141:K85;K88ab	-
43	CVCC 200	*E. coli*	O149:K91;K88ac	-
44	CVCC 1502	*E. coli*	O9:K88ac	+ +
45	CVCC 249	*E. coli*	O1	-
46	CVCC 1524	*E. coli*	O8:K91;K88ac	-
47	CVCC 1510	*E. coli*	O9:K88ac	-
48	CVCC 198	*E. coli*	O138:K88	-
49	CVCC 1521	*E. coli*	O117:K88ac	+
50	CVCC 1500	*E. coli*	O149:K88ac	-
51	CVCC 225	*E. coli*	O149:K91,K88ac	-
52	K XG14	*Klebsiella*		-
53	K XG19-2	*Klebsiella*		-
54	K XG29-2–1	*Klebsiella*		-
55	K XGE12	*Klebsiella*		-
56	K XG23	*Klebsiella*		-
57	K XG18-1	*Klebsiella*		-
58	K XG25-2–1	*Klebsiella*		-
59	ATCC 13311	*Salmonella*	Typhimurium	+ +
60	DHLM②	*Salmonella*	Typhimurium	-
61	SPu-116	*Salmonella*	Pullorum	-
62	ATCC 13076	*Salmonella*	Enteritidis	+

+++ Complete lysis, ++ Lysis, + Turbid lysis, – No plagues

rate for the first 3 h and then increased to 1.83 of OD_{600}. In contrast, infection with the phage at an MOI of 1, 0.1, 0.01, 0.001 or 0.0001 caused rapid cell lysis within 6 h, resulting in a 0.18 ~ 0.55 reduction of OD_{600} in cell concentration. In contrast, infection at an MOI of 0.00001 initially did not cause cell lysis within 2 h, but there was a rapid cell lysis during latter hours.

Comparative genomic analysis and evolution analysis

Based on sequence analysis, the genome of phage vE20 was consisted of 77,938 bp double-stranded DNA (dsDNA), with 121 ORFs (26 on the forward strand and 95 on the reverse strand) and a total G+C content of 42.17%. Among the 121 ORFs, 77 had significant homology to reported functional genes, while the remaining 44 ORFs were annotated as hypothetical proteins. As shown in Table 3, the functional ORFs were divided into 5 functional modules: DNA replication/ modification / regulation (ORFs 50, 72, 73, 108), phage morphogenesis (ORFs 9, 10, 13, 14, 18, 19), DNA packaging (terminase large subunit, ORFs 4, 5, 6) and phage lysis (ORF 16), and some additional proteins, without virulence-associated, antibiotic, lysogeny related genes. It suggested that vE20 is a virulent phage.

BLASTN analysis revealed that vE20 genome had the highest similarity with two phages: *Escherichia* phage ECBP2 (accession NC018859.1, 89.72% identity, and 86% query coverage) and *Escherichia* phage vB_EcoP_SU10 (accession No. NC027395.1, 75.89% identity, and 72% query coverage). The amino acid sequences of lysin coded by vE20 ORF16 were further used to construct a phylogenetic tree. As shown in Fig. 5A, fifteen closest relative phages were identified by Blast-P analysis. It showed that

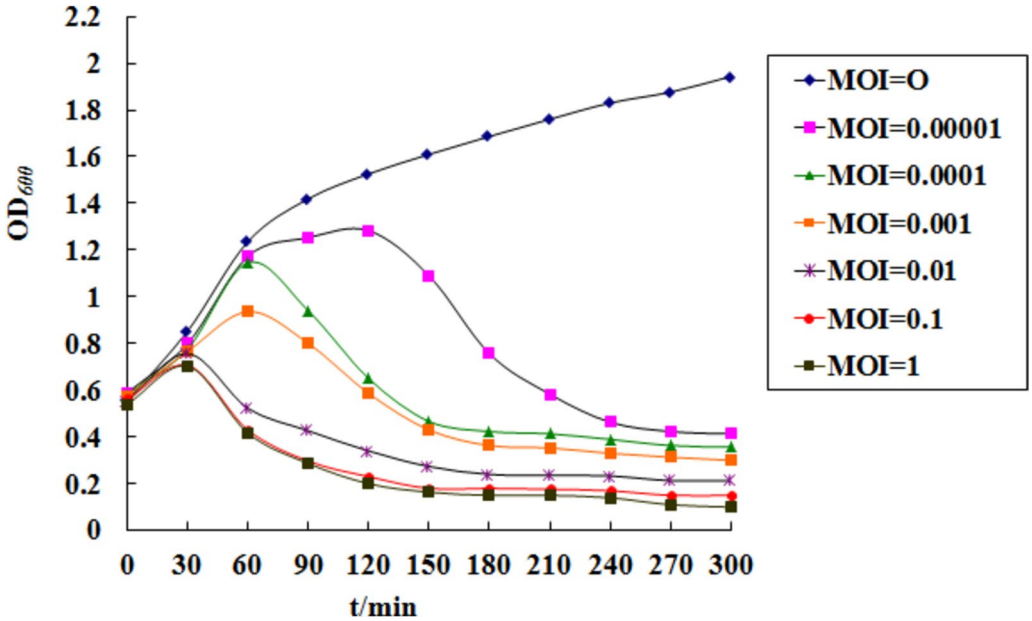


Fig. 4 The lysis rate of phage vE20 in different MOI

vE20 had the highest homology with *Escherichia* phage EK010 (accession No. BCG45030.1), and higher homology with *Podoviridae* sp. (accession No. DAL79791.1) and *Shigella sonnei* (EFV9058517.1). Furthermore, we construct a phylogenetic tree based on the nucleic acid sequence of the capsid protein (Fig. 5B). The phage vE20 had low homology with other phages.

Discussion

In recent times, the overuse of antibiotics has resulted in the emergence of multi-drug resistance against *E. coli*, which is negatively impacting food safety, human health, and the environment. The issue of bacterial resistance has gained significant attention, and researchers are now exploring new antibacterial solutions to address the problem. One such solution is phage therapy, which is becoming increasingly popular due to the bactericidal ability and bacterial host specificity of phages.

This study focused on the isolation and characterization of a new *E. coli* phage (vE20) from chicken samples. The phage was identified as a member of the *Podoviridae* families and was found to differ from most other *E. coli* phages, such as KFS-EC, with respect to head and tail length. (Lee et al. 2020) (head length: 100.25 ± 7.26 nm, tail length: 113.88 ± 10.19 nm), and close to phage SBSWF27 (Beheshti et al. 2015) (head length: 98 nm, tail length: 14 nm).

The one-step growth curve data showed that vE20 had a latent period of 10 min and a burst size of 60 PFU/cell, which is smaller than that of phages JS09 (79 PFU/cell) (Zhou et al. 2015) and CEV1 (150 PFU/cell) (Niu et al. 2012), but larger than others phage AR1 (34 PFU/cell) (Kutter et al. 2011). The latent period referred to the shortest time required for bacteriophages particles to adsorb and release progeny bacteriophages from infected host cells. Shorter latent period indicated that phages can infect host bacteria faster. Smaller burst size indicated the phages lower effect on infection. The comparation of vE20 and other phages showed vE20 had better infection than AR1, lower effect than JS09 and CEV1. Importantly, vE20 showcased highly effective lytic properties against *E. coli* strains that are resistant to multiple drugs.

Environmental conditions such as pH, temperature, and certain cations are known to affect the virulence of phages. In this study, it was observed that vE20 had an optimum MOI of 100, and it was effective in eliminating bacteria and producing phage progeny for subsequent cycles of phage lytic activity. Additionally, vE20 was found to survive a wide range of temperatures (30–60 °C) and pH (3–11). This resistance was comparable to that observed with several other phages analyzed in previous research (Chen et al. 2016; Lee et al. 2020; Shahin et al. 2019). As a promising candidate for biological pest control, bacteriophages must have high lysis ability. Phage vE20 is a new type of bacteriophage with high lytic activity against host strains. A wide host range is an important criterion for selecting promising phage candidates for effective Phage therapy. Therefore, further characterization of the cleavage ability of vE20 against various pathogens, including several *Salmonella* and *Klebsiella*

Table 3 Genomic annotation of functional ORF of phage vE20

ORF	Start	Stop	Length	Strand	Function
1	421	627	207	+	Phage protein
2	680	1342	663	+	Phage protein
3	1405	1872	468	+	Phage protein
4	1939	2421	483	+	Phage terminase, large subunit
5	2500	2802	303	+	Phage terminase, large subunit
6	2803	3480	678	+	Phage terminase, large subunit
7	3546	5789	2244	+	Phage portal (connector) protein
8	5799	6035	237	+	Phage protein
9	6035	7117	1083	+	Phage capsid and scaffold
10	7158	8213	1056	+	Phage capsid and scaffold
11	8246	9313	1068	+	Phage protein
12	9414	10160	747	+	Phage protein
13	10170	13391	3221	+	Phage tail fiber
14	13434	15590	2157	+	Phage tail fiber
15	15681	15899	209	+	Phage protein
16	15933	16424	492	+	Phage lysin
17	16437	17240	804	+	Phage baseplate (uncharacterized for now)
18	17252	20251	3000	+	Phage tail fiber
19	20299	21312	1014	+	Phage tail fiber
20	21314	22348	1035	+	Phage protein
21	22361	23158	798	+	Phage internal (core) protein
22	23168	24220	1053	+	Phage protein
23	24230	25198	969	+	Phage protein
24	25213	26880	1668	+	Phage protein
25	26951	31396	4446	+	Phage protein
26	31882	31616	267	-	hypothetical protein
27	32190	31891	300	-	Phage protein
28	32557	32153	405	-	Phage terminase, small subunit
29	32735	32544	192	-	Phage protein
30	33567	32746	822	-	Phage exonuclease
31	34156	33560	597	-	dNMP kinase
32	34806	34156	651	-	Phage protein
33	35144	34806	339	-	Phage protein
34	35317	35144	174	-	Phage protein
35	35816	35358	458	-	Phage-associated homing endonuclease
36	36181	35840	341	-	Phage protein
37	36981	36241	741	-	Phage protein
38	37935	37315	621	-	putative serine/threonine protein phosphatase
39	38095	37928	168	-	hypothetical protein
40	38282	38079	204	-	Phage protein
41	38506	38282	225	-	hypothetical protein
42	38688	38503	185	-	hypothetical protein
43	38842	38663	180	-	Phage protein
44	39018	38839	180	-	Phage protein
45	39407	39015	393	-	Phage protein
46	39688	39446	243	-	Phage protein
47	39858	39691	168	-	hypothetical protein
48	39989	39855	135	-	hypothetical protein
49	40238	40047	192	-	hypothetical protein

Table 3 (continued)

ORF	Start	Stop	Length	Strand	Function
50	42100	40256	1845	-	Phage DNA-directed DNA polymerase
51	42470	42117	354	-	hypothetical protein
52	42731	42474	258	-	hypothetical protein
53	42984	42733	252	-	hypothetical protein
54	43535	43041	495	-	hypothetical protein
55	43852	43532	321	-	Phage protein
56	44637	43900	738	-	Phosphate starvation-inducible protein PhoH, predicted ATPase
57	45136	44816	324	-	Phage protein
58	45339	45136	204	-	hypothetical protein
59	46161	45511	651	-	Thymidylate synthase ThyX
60	46621	46154	468	-	hypothetical protein
61	46844	46629	216	-	hypothetical protein
62	47064	46852	212	-	hypothetical protein
63	47341	47066	276	-	Phage protein
64	47519	47343	177	-	hypothetical protein
65	47698	47519	180	-	hypothetical protein
66	48329	47763	567	-	Phage integrase
67	48651	48322	330	-	Phage protein
68	48928	48722	207	-	hypothetical protein
69	49125	48928	195	-	hypothetical protein
70	49677	49144	534	-	Deoxycytidine triphosphate deaminase
71	51134	49674	1461	-	hypothetical protein
72	51685	51137	549	-	DNA polymerase, phage-associated
73	53469	51679	1791	-	Phage DNA primase/helicase
74	53711	53505	207	-	hypothetical protein
75	54132	53698	435	-	Phage protein
76	54661	54125	537	-	HNH homing endonuclease Phage intron
77	55145	54903	243	-	Phage protein
78	56412	55177	1236	-	Phage protein
79	56636	56415	222	-	Phage protein
80	56850	56617	234	-	Phage protein
81	58865	56862	2004	-	Phage protein
82	60073	58973	1101	-	Phage protein
83	60696	60070	627	-	Phage protein
84	61512	60706	807	-	Phage protein
85	62455	61505	951	-	Phage protein
86	63681	62464	1218	-	Phage protein
87	64055	63693	363	-	Phage protein
88	64219	64052	168	-	hypothetical protein
89	64709	64461	249	-	hypothetical protein
90	64942	64709	234	-	Phage protein
91	65383	64970	414	-	hypothetical protein
92	65569	65453	111	-	Phage protein
93	65840	65583	258	-	hypothetical protein
94	66093	65830	264	-	hypothetical protein
95	66351	66097	255	-	hypothetical protein
96	67109	66855	255	-	hypothetical protein
97	67437	67111	327	-	Phage protein
98	67533	67459	75	-	tRNA-Arg-TCT

Table 3 (continued)

ORF	Start	Stop	Length	Strand	Function
99	67752	67543	210	-	hypothetical protein
100	67925	67761	165	-	hypothetical protein
101	68358	68101	258	-	Phage protein
102	68606	68355	252	-	hypothetical protein
103	68825	68616	210	-	hypothetical protein
104	69049	68909	141	-	hypothetical protein
105	69660	69352	309	-	hypothetical protein
106	69891	69661	231	-	hypothetical protein
107	70064	69888	177	-	hypothetical protein
108	70353	70048	306	-	DNA helicase, phage-associated
109	70941	70555	387	-	Phage protein
110	71702	71160	543	-	Phage protein
111	71982	71773	210	-	hypothetical protein
112	72204	71983	222	-	hypothetical protein
113	72425	72201	225	-	hypothetical protein
114	73436	72534	903	-	Phage protein
115	74855	75238	384	+	hypothetical protein
116	75660	75334	327	-	phage protein
117	75763	75671	93	-	hypothetical protein
118	76032	75760	273	-	hypothetical protein
119	76283	76035	249	-	hypothetical protein
120	77143	76280	864	-	Phage protein
121	77688	77539	149	-	hypothetical protein

isolates, will expand the therapeutic potential of this highly virulent phage. Compared to antibiotics, bacteriophages have a narrower antibacterial spectrum. Usually, one bacteriophage can only eliminate one type of bacteria. Different *E. coli* phages can infect different *E. coli* strains. In this study, vE20 has a wide host range and can infect not only *Escherichia coli* but also other *Salmonella*. In other studies, they mainly studied the host range of *Salmonella* bacteriophages against foodborne *E. coli*, with less research on the lysis effect of *E. coli* bacteriophages on *Salmonella*. Bacteriophages are mainly used in livestock and poultry products, as well as in food, to kill bacteria through spraying. Spraying of phages has good antibacterial and preservation effects in egg milk and poultry meat products, but has no effect on the flavor or appearance of food. The results of this study indicate that vE20 has greater potential as a new type of food sterilization product.

Genome-wide sequencing and genome annotation of vE20 showed that it has a 77,938 bp long genome, 121 ORFs without virulent genes and a total G + C content of 42.17%. The predicted homology of whole- genome genes showed that vE20 were most similar to phage EBP2 (accession No. NC 18859.1). In previous study (Nho et al. 2012), ECBP2 has 77, 135 bp long genome, 120 ORFs with GC content of 42.42%. Phage vE20 has all the basic structural diagrams and functional genes that encode host lysis proteins, DNA replication/ modification/ regulation and packaging proteins, tail structural proteins, and some additional functional proteins. Besides, the phylogenetic tree analysis of the lysin proteins encoded by ORF16 showed that vE20 had high homology with *Escherichia* phage EK010, *Podoviridae* sp. and *Shigella sonnei*. This indicates that the genomes of vE20 and EK010 are similar and have a close evolutionary relationship, possibly originating from the same ancestor. Furthermore, the phylogenetic tree analysis of the capsid protein showed that vE20 had low homology with other phages. It indicated that vE20 may be a new type of phage.

Most literature reports that the use of bacteriophages is safe and reliable, and there is currently no clear report on the side effects of using bacteriophages. However, bacteriophages themselves are a virus, and the safety of their use in poultry farming production needs further research and certification.

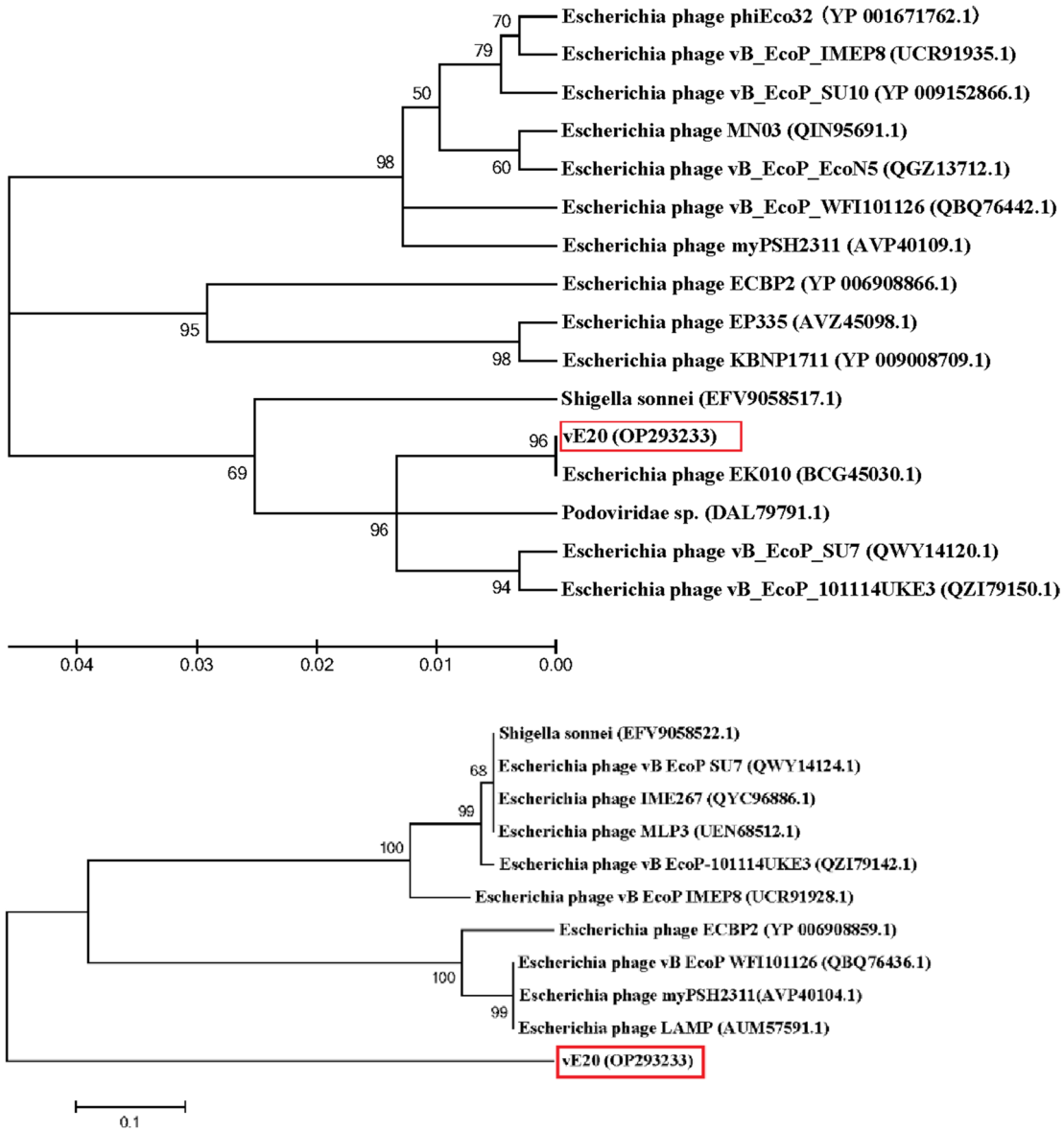


Fig. 5 The phylogenetic tree of Lysin (A) and Capsid protein (B) in phage vE20. Note: The GenBank accession numbers are also provided with phage names. The trees showed the relationship of Lysin or Capsid protein of phage vE20 with other closely and distant phages

Conclusions

The lytic phage vE20 was isolated from chicken and well characterized regarding physiological and genetic aspects. This lytic phage was possessed of broad host range, rapid lytic cycle, broad range resistance to temperature and pH, high burst size and the absence of any undesirable genes such as lysogeny, virulence factors, and antimicrobial resistance genes. And the phage has high bactericidal activity against *E. coli* EXG20-1. Therefore, vE20 has the potential to be a novel natural biocontrol agent for *E. coli* in food products.

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Authors' contributions
ZZ, YW and HL done the experiments and analyzed the experimental data. ZZ and HB wrote this article. HZ and YZ and RW contributed to revise the manuscript. All authors approved the final version.

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Availability of data and materials
The complete genome sequence of phage vE20 was deposited at GenBank under accession number OP293233.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their consent for publication.

Competing interests
All authors declares that they have no conflict of interest.

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