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Studies on physical characteristics of phages against *Escherichia coli* and whole genome sequencing and analysis of a coliphage

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Abstract

The present study is focused on isolation of phages against *Escherichia coli.* The study revealed that out of 30 sewage samples from 12 dairy farms, 11 coliphages were isolated. All the 11 phages were characterised and on the basis of morphology plaque, sizes ranging 1–8 µm in diameter were observed. All the phages were stable at temperatures of 50 °C and 60 °C for 48 h but were inviable at 65 °C > 6 h of exposure. Upon UV exposure for 30 min, all the phages were stable but with reduced viability. Whole genome sequencing of a phage showed 97.58% identity to *Escherichia* phage vB EcoM-ECP26, complete genome. Also phage contigs were identical to antibiotic resistance genes for β-lactamases, tetracyclins, glycopeptides, macrolides, diaminopyrimidines, aminoglycosides and carbapenems in abundance. Thus, it could be concluded from the study that phages harbour various antibiotic resistant genes that could lead to transmission of antibiotic resistance in the host, i.e. bacteria.

Antibiotic resistance, bacteria, genes, sequencing

Coliphages are a type of bacteriophage that attack *Escherichia coli* and are often present in the gut. They are discharged in the faeces of warm-blooded animals and can be found in high concentrations in raw wastewater. Many other matrices contaminated with faecal remnants also harbour these phages (Ewert and Paynter 1980). As per the type of infection, there are two types of coliphages i.e., lytic and lysogenic. Lytic phages overtake the host cell machinery to produce new phages and lysogenic phages integrate phage DNA into the host genome known as prophage. The lysogenic life cycle can revert back to the lytic form. Prophages can regulate gene expression and function, change host cell physiology by introducing new functions or altering pre-existing ones like production of virulence factors (Hargreaves et al. 2014; Feiner et al. 2015). Temperate phages, can facilitate the transfer of bacterial DNA that potentially confers new phenotypes such as antibiotic resistance (Davies-Colley et al. 2005). The genome of coliphages consists of either double-stranded (ds) or single-stranded (ss) DNA or RNA with a protein capsid and a lipid envelope in some cases.

Coliphages can be categorised into seven major morphological groups; four of which contain somatic coliphages and three of which contain F-specific coliphages (Cole et al. 2003; Mesquita et al. 2010). Somatic coliphages are members of the families *Myoviridae*, *Siphoviridae*, *Podoviridae*, and *Microviridae*. *Microviridae* have circular ds DNA genome whereas other family members have linear ds DNA genome (Grabow 2002). F-specific coliphages belong to *Inoviridae*, *Leviviridae*, and *Tectiviridae* families of bacteriophages (De Paepe and Taddei 2006; Jones et al 2017). Phages of *Inoviridae* family have ssDNA as their genome whereas phages of *Tectiviridae* family have dsDNA as their genome. Members of *Leviviridae* family have ssRNA as their genome (Mesquita et al. 2010). *Myoviridae*, *Siphoviridae*, and *Podoviridae* are the families of tailed

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phages categorised under the order Caudovirales. Tailed phages represent the majority of existing bacteriophages. Phages of the *Myoviridae* family have complex contractile tails, *Siphoviridae* have long non contractile tails, and *Podoviridae* have short non contractile tails (Cole et al. 2003).

The size of coliphage genome varies enormously, ranging from 3.39 kb to 386.44 kb (Chinnadurai et al. 2018). The guanine cytosine (GC) content of most of the coliphages lies within 35.3–60.0% (Olsen et al. 2020). Comparative analysis of phage genomes has revealed that different segments of genome have discrete evolutionary histories which occurs due to the horizontal genetic exchange (Hatfull and Hendrix 2011).

There are a number of studies stating that phages play an important role in the origin and dissemination of antibiotic resistance genes by transduction, and that these genes may confer resistance to several antibiotics, including tetracycline, ampicillin, and bleomycin (Balcazar 2014). Therefore, the present study was envisaged to isolate phages against *E. coli* from the environment with an aim to identify antibiotic resistance genes/gene cassettes in the coliphage.

Materials and Methods

Coliphage isolation

A total of 30 sewage slurry samples (80–90 ml) were collected from 12 dairy farms in Punjab and processed for the isolation of phages in laboratory. In brief, slurry samples were centrifuged at 4000 × *g* for 10 min and supernatant was collected. To the supernatant (40 ml), 10 ml of fresh log phase *E. coli* (Microbial Type Culture Collection 901) of optical density OD_{600} 0.5–0.7 grown in tryptose soy broth were mixed with 50 ml of double strength NZ amine casamino yeast magnesium sulphate (NZCYM) broth and incubated on a rotatory shaker at 37 °C for 48 h. After incubation, 10 ml of supernatant were aspirated and centrifuged at 6000 × *g* for 15 min and were passed through a sterile 0.45 µm polyvinylidene fluoride (PVDF) syringe filter first, and further through a sterile 0.22 µm PVDF syringe filter. The filtrate was designated as bacteria-free filtrate (BFF) and kept at 4 °C.

Double agar overlay technique for phage isolation was used (Chandra et al. 2011). Five ml of semisolid NZCYM agar (NZCYM broth with 0.75% of agar) were melted and kept at 55 °C and 100 µl of different serially diluted BFF (× 10) were mixed with equal concentration of log phase *E. coli* (OD_{600} 0.5–0.7) and after mixing were spread evenly on the surface of 1.5% NZCYM agar plate and allowed to solidify and incubate at 37 °C for 18–24 h. The following day, plates showing presence of plaques, i.e., clearing of bacterial lawn by phages, were selected for isolation of coliphages.

Secondary streaking

A well isolated plaque picked from above using straight wire was streaked horizontally and vertically in lines across the plate NZCYM plates with bacteria and incubated at 37 °C for 24 h for the lysis by phages along the streak lines.

Elution and concentration of bacteriophages

Four ml of sodium chloride-magnesium sulphate (SM) buffer were poured over the plate with secondary streaking and disturbed with the help of the straight inoculation wire and plate was kept at 4 °C overnight. The next day, elute from the plate was collected and centrifuged at 6,000 × *g* for 15 min. The supernatant was passed through a 0.22 µm filter, and the filtrate was designated as concentrated phage preparation (CPP) and stored at 4 °C.

Enumeration of plaque forming unit plaque forming unit

For the phage enumeration, tenfold serial dilution (up to 10^{-11}) of CPP was prepared for determination of the plaque forming unit (PFU). From each dilution, 100 µl of phage suspension were taken and plated as per double agar overlay protocol. Following the incubation of agar plates, the plaques formed on each plate were counted and PFU was calculated using following formula:

PFU/ml = no. of plaques × dilution factor / volume of phage plated

Characterization of coliphages

The lytic activity of phages was tested against heterologous host viz. *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp as well as homologous host i.e., *E. coli* (10) field isolates. The plaques were evaluated for their shape, size, and form (turbidity) on lawn culture of *E. coli* (Jurczak-Kurek et al. 2016).

Physical characterization of phages

The phages were exposed to different temperatures viz. 50 °C, 60 °C and 65 °C for a period of 48 h. After the completion of incubation at the desired temperature, aliquots (100 µl) of the phages were subjected to phage PFU/ml estimation at 3 h, 6 h, 9 h, 24 h, and 48 h to determine the survivability of phages.

To study the effect of UV light, the phages were exposed to UV light (254 nm) in a biosafety cabinet for different time intervals of 10, 20, and 30 min. After the completion of exposure, aliquots (100 µl) of the phages were subjected to phage PFU/ml estimation to determine survivability of the phages.

DNA extraction of phages

For the extraction of DNA, phage precipitation solution (30% polyethylene glycol 8000, 3.3 M NaCl) at the ratio of 1:0.4 was added to CPP and mixed gently by inverting tubes. After overnight incubation on ice, it was centrifuged at 10,000 × *g* for 30 min at 4 °C and the supernatant was discarded. The pellet was dried at room temperature and resuspended in one ml of SM buffer and filtrated through a 0.22 µm filter (Rai et al. 2019). This filtrate was used for DNA isolation using Phage DNA Isolation Kit (Norgen Biotek Corporation, Canada) according to manufacturer's protocol.

The purity and concentration of the extracted genomic DNA was estimated using a Nanodrop (Thermo Scientific, Waltham Massachusetts, USA). The optical density (OD) at 260 nm and 280 nm of the sample was measured by using nuclease free water as blank and the ratio of 260/280 was calculated. A ratio of 1.6–1.8 was considered satisfactory.

Whole genome sequencing of a phage

The genomic DNA of a coliphage was subjected to paired-end sequencing (2 × 150 bp). DNA libraries were prepared using NEB Next Ultra II FS DNA Library Prep Kit (NEB, Ipswich, Massachusetts) according to manufacturer's protocol. The libraries were pooled and sequenced on Novaseq 6000, Illumina platform (Lifecell Technologies, Bengaluru, India). A quality check was performed on generated raw data using FastQC and MultiQC software. The data were checked for base call quality distribution, percentage of bases above Q30, GC percentage, and sequencing adapter contamination. Pre-processing of data was carried out before data analysis to remove adaptor sequences and low-quality bases using fastp v0.12.4. The QC passed reads were processed using Kraken2 v2.1.2, which classified the reads using k-mer based homology against the mini National Centre for Biotechnology Information (NCBI) nucleotide database of sequences (containing bacteria, viruses, archaea and fungi).

The reads were assembled to contigs using MetaviralSPAdes v3.15.4 with k-mer sizes 21, 33, 55, 77, 99 and 121. The assembly quality was checked by mapping the reads back onto the assembled contigs using bowtie2 v2.4.5. The genome completeness was checked using CheckV v1.0.1. Assembled contigs were annotated by BLAST v2.13.0 homology search against NCBI nucleotide database and by using Prokka v1.13-Viruses option. The predicted proteins were then annotated by BLAST search against the Uniref100 database. Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Clusters of Orthologous Groups of proteins (COG) database annotations were obtained by submitting the protein sequences of the sample to the KEGG Automatic Annotation Server and WebMGA (software for metagenomic data analysis), respectively. The whole genome sequences were uploaded to NCBI for obtaining Accession Number.

Results

Isolation of phages

A total of 30 sewage slurry samples collected from 12 dairy farms were processed for isolation of coliphages using double agar overlay method and 11 out of 30 bacterial free filtrates, showed plaque formation. For isolation and purification, a single plaque was selected, picked using secondary streaking (Table 1).

Characterisation of phages

The isolated phages were tested for their ability to lyse field isolates of *E. coli* (10) and all phages lysed all the field isolates. However, on heterologous host, none of the phages was able to lyse *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Morphological characterisation revealed that most of the phages formed clear round plaques while some formed turbid plaques. Some phages displayed greater turbidity toward plaque peripheries (bull's eye appearance) and the plaque size ranged between 1–8 mm.

Table 1. Isolation of phages from dairy farms.

Sample	Location	Dairy farm (n. of sewage samples collected)	N. of phages isolated (phage name)
1	Ludhiana	1 (5)	2 (B1, B2)
		2 (2)	1 (B3)
		3 (2)	1 (B4)
		4 (2)	1 (B5)
2	Jalandhar	5 (3)	0 (0)
		6 (3)	1 (B6)
3	Moga	7 (3)	1 (B7)
		8 (2)	0 (0)
4	Raikot	9 (2)	1 (B8)
		10 (2)	0 (0)
5	Shahkot	11 (2)	2 (B9, B9.1)
6	Jagraon	12 (2)	1 (B10)
	Total	12 (30)	11

Physical characterisation of phages

All the phages exposed to temperatures viz., 50 °C, 60 °C and 65 °C for up to 48 h revealed that at 50 °C, there was gradual reduction in phage number till 48 h of exposure. Most of the phages survived (90–93%) after 9 h of exposure. After 48 h of exposure, the survival rate was 82–85% except for three phages where it was 61.26% (B8), 62.57% (B5) and 68.67% (B4). Among all the phages, phage B2 was found to be thermally most stable with the survival rate of 87.7% (Table 2).

At 60 °C, a reduction of 8–16% in the phage number was observed. After 48 h of exposure, the survival rate was 61–79%. Among all the phages, B6 (40.14%) and B8 (40.19%) were found to be the most sensitive to this temperature (Table 2).

At 65 °C most of the phages could tolerate this temperature up to maximum 6 h while three phages (B2, B4 and B6) could survive only up to 3 h (Table 2).

Effect of UV light

Phages exposed to UV light (254 nm) for 10, 20, and 30 min exhibited a reduction of 10–12%, 15–20% and 25–30%, respectively (Table 3).

Whole genome sequencing of the phage

A total of 22977722 raw sequence reads were assembled to contigs using MetaviralSPAdes (v3.15.4) yielded a genome of 136047 (136 KB) bp in length with 43.61% GC content. The coliphage genome sequence was submitted to NCBI with the accession number OQ420740. The assembled genome was annotated and visualised as circular map by Proksee (Plate III, Fig. 1).

The assembled contigs were annotated using by BLAST v2.13.0 and by using Prokka v1.13. The summary of Prokka annotation is presented in Table 4. It was observed that a total of 206 CDS and 216 genes were identified. The predicted proteins were then annotated by BLAST individually search against the Uniref100 database.

Blast-n analysis of the assembled genome revealed that it was having 97.58% identity to *Escherichia* phage vB EcoM-ECP26, complete genome (Accession no: MK883717.1). This phage belonged to class: *Caudoviricetes* (tailed bacteriophages with dsDNA), subfamily: *Vequintavirinae*, and genus: *Vequintavirus*.

Table 2. Effect of temperature on phages.

Phage		Phage concentration (log10) at various time intervals (h)						
	Temperature (°C)	0	3	6	9	24	48	% Reduction (48 h/0 h)
B 1	50	11.87	11.65	11.36	11.00	10.62	9.85	82.98
	60	11.87	10.81	9.96	8.90	7.00	5.41	45.50
	65	11.54	6.92	4.62	0	0	0	100
B 2	50	9.84	9.81	9.25	9.20	8.81	8.63	87.77
	60	9.84	9.34	8.84	8.30	7.85	6.47	66.02
	65	10.09	3.47	0	0	0	0	100
B 3	50	10.60	10.30	10.11	9.94	9.25	9.02	85.09
	60	10.25	9.38	9.20	8.90	8.90	8.30	80.97
	65	10.39	5.85	3.33	0	0	0	100
B 4	50	10.79	8.92	8.65	7.92	7.66	7.41	68.67
	60	10.79	8.04	7.41	6.73	5.70	4.80	44.48
	65	9.11	4.90	0	0	0	0	100
B 5	50	10.50	9.79	8.96	7.50	6.97	6.57	62.57
	60	10.50	8.71	7.90	6.91	5.90	4.80	45.71
	65	9.90	5.68	3.75	0	0	0	100
B 6	50	9.66	8.85	8.69	8.00	7.47	7.04	72.87
	60	9.67	8.67	8.00	7.77	5.60	3.95	40.14
	65	9.67	5.21	0	0	0	0	100
B 7	50	10.25	9.87	9.41	9.14	8.76	8.50	82.92
	60	10.25	9.90	8.80	8.04	7.70	6.69	65.26
	65	9.95	5.80	4.51	0	0	0	100
B 8	50	10.43	9.20	8.81	7.32	6.90	6.39	61.26
	60	10.43	8.34	7.81	7.54	5.90	4.41	40.19
	65	10.43	6.33	3.39	0	0	0	100
B 9 and B 9.1	50	10.78	10.59	10.27	10.26	9.20	9.00	83.48
	60	10.56	9.49	9.04	8.93	8.76	8.30	78.50
	65	10.25	6.81	3.77	0	0	0	100
B 10	50	11.00	10.49	10.17	9.95	9.34	9.04	82.18
	60	11.00	9.30	9.07	8.75	8.00	5.90	53.63
	65	10.92	6.70	3.60	0	0	0	100

Table 3. Effect of UV (254 nm) light on phages.

		Phage concentration (log10)				
Sample	Phage	0 min	10 min	20 min	30 min	% Reduction (30 min/0 min)
1	B 1	10.94	8.96	7.60	6.81	62.24
2	B 2	9.87	8.88	7.90	7.39	74.87
3	B 3	10.43	9.97	9.43	7.96	76.31
4	B 4	9.96	8.85	7.92	6.84	68.67
5	B 5	10.41	9.57	8.41	7.63	73.29
6	B 6	9.67	8.56	8.07	7.57	78.28
7	B 7	10.95	9.95	9.51	8.13	74.24
8	B 8	10.43	9.17	8.38	7.61	72.90
9	B 9 and B 9.1	10.04	8.85	7.60	7.00	67.30
10	B 10	9.95	8.73	7.70	6.95	69.84

Table 4. Functions of predicted annotated proteins in phage genome (Accession n. OQ420740).

Sample	Query I.D.	Annotation (blast p)	Function
1	B9-1_00003	Anaerobic NTP reductase small subunit	DNA synthesis
2	B9-1_00005	Anaerobic ribonucleoside triphosphate reductase	DNA synthesis
3	B9-1_00006	Glutaredoxin	Post translational modification, chaperones
4	B9-1_00007	Ribonucleotide reductase, R2/beta subunit	DNA synthesis
5	B9-1_00008	Ribonucleoside-diphosphate reductase large subunit	DNA synthesis
6	B9-1_00011	Thymidylate synthase, flavin-dependent	DNA synthesis
7	B9-1_00017	Putative DNA N6-adenine methyltransferase	Protection of DNA during synthesis
8	B9-1_00028	Glycoside hydrolase family 104 protein similar to Escherichia virus P2 endolysin	Host cell lysis
9	B9-1_00029	ATP-dependent DNA ligase	DNA replication and repair
10	B9-1_00033	NAD-dependent protein deacetylase of SIR2 family	Regulate gene silencing, DNA repair, metabolic enzymes, and life span.
11	B9-1_00037	Metallophosphoesterase family protein	Regulation of transcription
12	B9-1_00038	Putative transcriptional regulator protein	Regulation of transcription
13	B9-1_00055	I-spanin	Phage lysins
14	B9-1_00056	O-spanin	Phage lysins
15	B9-1_00057	Terminase, large subunit	DNA translocation and packaging
16	B9-1_00059	Peptidase_S78_2 domain-containing protein	Capsid maturation
17	B9-1_00060	Head stabilization/decoration protein	Capsid stability
18	B9-1_00061	Putative head stabilization/decoration protein	Capsid stability
20	B9-1_00062	Major capsid protein	Stability of DNA in phage head
21	B9-1_00068	Tail sheath protein	External contractile envelope of tail
22	B9-1_00071	Tape measure protein	Tail assembly
23	B9-1_00073	Structural protein	Structural protein
24	B9-1_00075	Gp138_N domain-containing protein	Infection of host
35	B9-1_00077	Tail_spike_N domain-containing protein	Recognition and binding to host
36	B9-1_00078	Tail fibre protein	Recognition of bacterial surface
37	B9-1_00079	Tail fibre protein	Recognition of bacterial surface
38	B9-1_00084	DUF1983 domain-containing protein	Host specificity
39	B9-1_00085	Base plate component	Cell binding and genome injection
40	B9-1_00088	Tail fibre protein	Recognition of bacterial surface
41	B9-1_00089	Caudo_TAP super family	Tail assembly
42	B9-1_00091	Tail fibre protein	Recognition of bacterial surface
43	B9-1_00093	Tail fibre protein	Recognition of bacterial surface
44	B9-1_00094	Bacterial Ig-like domain (group 2)	Surface proteins
45	B9-1_00107	Anti-sigma factor	Inhibition of transcription
46	B9-1_00108	Anti-sigma factor	Inhibition of transcription
47	B9-1_00109	Alpha 1-3 fucosyltransferase	Cellular activities
48	B9-1_00115	MoxR ATPase	Cellular activities
49	B9-1_00117	Hydrolase_2	Cell wall hydrolysis
50	B9-1_00118	rllA protector	Inhibition of lysis
51	B9-1_00119	rllB lysis inhibitor	Inhibition of lysis
52	B9-1_00120	ATP-dependent RecD-like DNA helicase	Replication, recombination, and repair
53	B9-1_00122	Adenine methyltransferase	Replication, recombination, and repair
54	B9-1_00127	RecA-like domain of Gp4D helicase	DNA replication
55	B9-1_00128	DNA polymerase superfamily A	Replication and repair
56	B9-1_00143	Macro domain protein	Conserved protein
57	B9-1_00174	DNA translocase	DNA synthesis
58	B9-1_00190	Sigma 54 modulation factor	Cellular activities
59	B9-1_00192	Sigma 54 modulation factor	Cellular activities
60	B9-1_00214	ATP-dependent Clp protease proteolytic subunit	Transcriptional regulator
61	B9-1_00215	PhoH-like protein	Phosphate metabolism
62	B9-1_00216	PhoH-like protein	Phosphate metabolism

The COG annotations were obtained by submitting protein sequences to WebMGA. The COG database classified proteins into different functional groups as depicted in (Plate III, Fig. 2).

From the predicted proteins outcome, it was observed that out of 216 genes, 132 were annotated as uncharacterised proteins, 17 as hypothetical proteins while, 61 genes were associated with phage structural components, phage replication, cell lysis, phage assembly etc (Table 4).

Presence of antibiotic resistance genes in the phage genome

The protein sequences of the phage were processed using the Resistance Gene Identifier (RGI) program using Comprehensive Antibiotic Resistance Database (CARD) and both strict and lose parameters were used for identification in the assembled genome. With stringent parameters, no antibiotic genes were detected. When loose parameters were used for detection, many antibiotic resistance gene fragments belonging to different class of antibiotics like β-lactams, tetracycline, fluoroquiolones, aminoglycocydes, macrolides, monobactams, lincosamides etc. were found (Table 5, Fig. 3, Plate IV).

Among genes for tetracycline resistance, *tetA* had sequence similarity to three viral contigs, while *tet (33)* was similar to two phage contigs. Seven phage contigs were found identical to *AAC (6')* gene which encoded for acetyl-CoA-dependent N-acetyltransferases. Two phage contigs harboured sequence similarities to QnrB16 and QnrB 72 genes for fluroquinolones resistance. Similarly, there were other phage contigs which were found

Table 5. Details of antibiotic resistance genes identified in phage genome (Accession n. OQ420740).

Sample	Class of drug	Antibiotic resistance genes	Mechanism of resistance
1	β-lactams	OXA-478, OXA-351, OXA-209, OXA-299, OXA-542, OXA-926, OXA-29, OXA-154, OXA-576, OXA-949, blaR1, CTX-M-13, MOX-7	Antibiotic inactivation
2	Tetracycline	tetA(46), tet(40), tet(38), tet(33),	Antibiotic efflux
		TxR, tet(36), tetL, tetK, tetW tetM	Antibiotic target protection
3	Macrolides	MsrC, vmlR	Antibiotic target protection
		ErmT, ErmN, Erm(O)-lrm, emtA	Antibiotic target alteration
		oleD, EreD, mphM	Antibiotic inactivation
		mphK	macrolide phosphotransferase (MPH)
		adeJ	Antibiotic efflux
4	Monobactams	PDC-331, PER-3	Antibiotic inactivation
5	Fluroquinolones	QnrB16	antibiotic target protection
6	Aminoglycosides	APH(2")-IIa, APH(6)-Id, AAC(6')-Ib8, AAC(3)-IXa, aacA43	Antibiotic inactivation
7	Aminocoumarin antibiotic	mdtB, mdtC	Antibiotic efflux
8	Glycopeptide antibiotic	vanTC, vanXB, vanRB, vanSE	Antibiotic target alteration
9	Lincosamides	LlmA 23S ribosomal RNA methyltransferase	Antibiotic target alteration
		lmrD	Antibiotic efflux
10	Sulphonamides	sul3	Antibiotic target replacement
11	Glycopeptides	vanTC, vanXB, vanRB, vanSE	Antibiotic target alteration
12	Carbapenems	KPC-29, SHV-216	Antibiotic inactivation
13	Diaminopyrimidines	dfrA23, dfrC	Antibiotic target replacement
14	Streptogramins	vgbA, vgbC	Antibiotic inactivation

identical to many other antibiotic resistance genes like genes for macrolide resistance (*ErmT, ErmN, Erm(O)-lrm, emtA*), glycopeptide antibiotics resistance (*vanTC, vanXB, vanRB, vanSE*), carbapenems resistance (*KPC-29, SHV-216*) and diaminopyrimidines resistance (*dfrA23, dfrC*).

Discussion

Phages represent the most abundant ‘life’ forms on Earth, with an estimated 10^{32} bacteriophages on the planet (Wommack and Colwell 2000). They are immensely diverse in morphology, size, and genomic organization. In the present study of 30 samples tested for phages against *E. coli*, eleven phages could be isolated. All the phages were tested for their ability to lyze homologous as well as heterologous host and it was observed that all the phages could not lyze heterlogous host but lyzed homologous field isolates effectively. The above findings were similar with those of Jofre et al. (2016) who have reported that bacteriophages are extremely host-specific and often only infect one bacterial species, or even certain strains within a species. It was revealed in the present study that most of the phages formed clear round plaques, typical for virulent phages, while some formed turbid plaques indicating lysogeny. Turbidity of plaques is a very common phenomenon and reasons for this could be due to lysis inhibition phenomenon shown by T-even phages or decreased lytic efficiency caused by aging of the bacterial lawn culture (Abedon 2009). In the present study, plaque size ranging between 1–8 µm in diameter was observed which is also similar to various other studies (Chandra et al. 2011).

All the phages exposed to different temperatures viz., 50 °C, 60 °C, and 65 °C for 48 h revealed that overall, phages could withstand temperatures of up to 60 °C with definite number reduction but exposure at 65 °C proved to be deleterious for the phages’ survival and they could withstand up to 6 h maximum post incubation. Similar results of lesser survivability of phages when exposed to higher temperatures and for a longer time have been observed by various authors (Ullah et al. 2021; Mishra et al. 2022; Abdelrahman et al. 2022). Phages and their hosts interact and evolve in the same environment which influences their survival in extreme environments. Acquisition of several mutations in structural proteins could be the reason for thermal stability of phages at high temperature (Jurczak-Kurek et al. 2016).

Effect of UV light on survival of phages

Phages exposed to UV light (254 nm) for 10, 20, and 30 min exhibited a reduction of 10–12%, 15–20% and 25–30% respectively. The findings were similar with various other findings (Megha et al. 2017). It has been observed that UV radiation can affect phages due to the formation of thymine dimmers leading to 5% loss in count of viable phages per hour due to UV light (Wommack 1999).

Whole genome sequencing of coliphages

On the basis of whole genome sequencing of a phage it was observed that major capsid protein E, structural proteins like tail fibre proteins, tape measure protein, tail spike protein, tail sheath protein, endolysins, spanin (O-spanin and I-spanin), terminase, PeptidaseS78_2 (domain-containing protein) were found in the genome. The phage genome possessed endolysin which had 98.72% identity to lysozyme of *Escherichia* phage vB EcoM SYGMH1. O-spanin and I-spanin was found similar to *Escherichia* phage vB EcoM-ECP26 (100%) and *Escherichia* phage vB EcoM-ECP32 (99.24%), respectively. Large subunit of terminase was identified in the genome and had 99.42% identity to *Escherichia* phage FV3 and *Escherichia* phage FFH2. The above findings are similar to the findings of various other authors who also reported presence of these in the phage genome (Lin et al. 2022).

Presence of antibiotic resistance genes in phage genome

The protein sequences of the phage were processed using RGI program which used CARD and in the present study, revealed phage contigs identical to antibiotic resistance genes for β-lactamases, tetracyclins, glycopeptides, macrolides, diaminopyrimidines, aminoglycosides and carbapenems were found in abundance. The findings of the present study are in complete agreement with the study by Moon et al. (2020) who also identified viral contigs that harboured genes for β-lactamases, aminoglycosides, vancomycin and diaminopyrimidines resistance. The results of our study indicate that a genetic exchange occurs between phages and bacteria that are present in environmental settings with antibiotics, as the samples for phage isolation were collected from farms that were using antibiotics for treating mastitis. Genomes of phages are a potential reservoir of antibiotic resistance genes as evident by analysis of the whole phage genome. These phage genomes with more antibiotic resistance genes pose a major threat as they play important role in dissemination of antibiotic resistance genes. Therefore, further studies focused on analysis of the phage genome from various sources are warranted.

Conflict of interest

The authors declare no conflict of interest.

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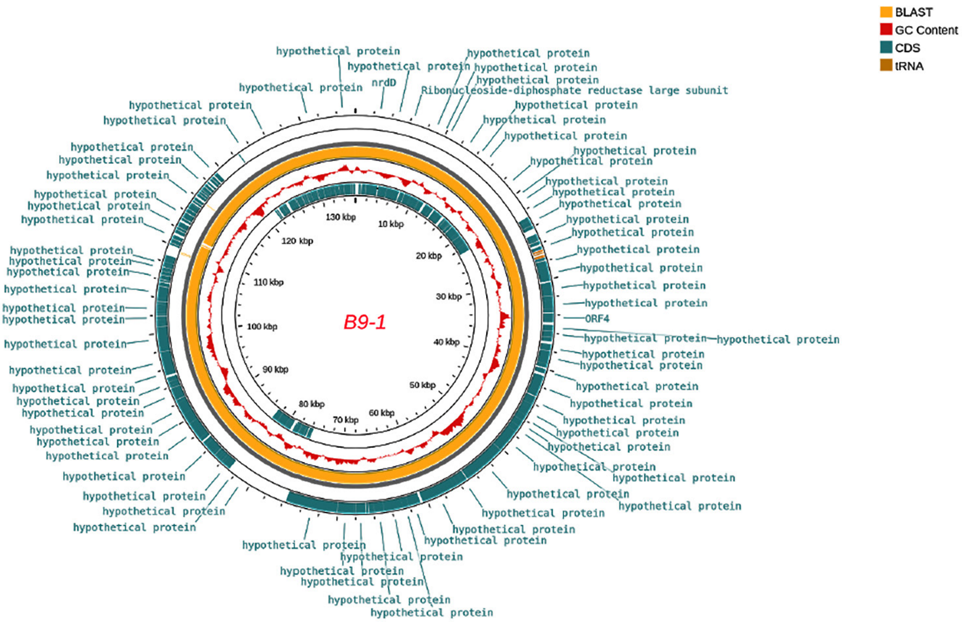

Fig.1. Circular map of assembled phage genome

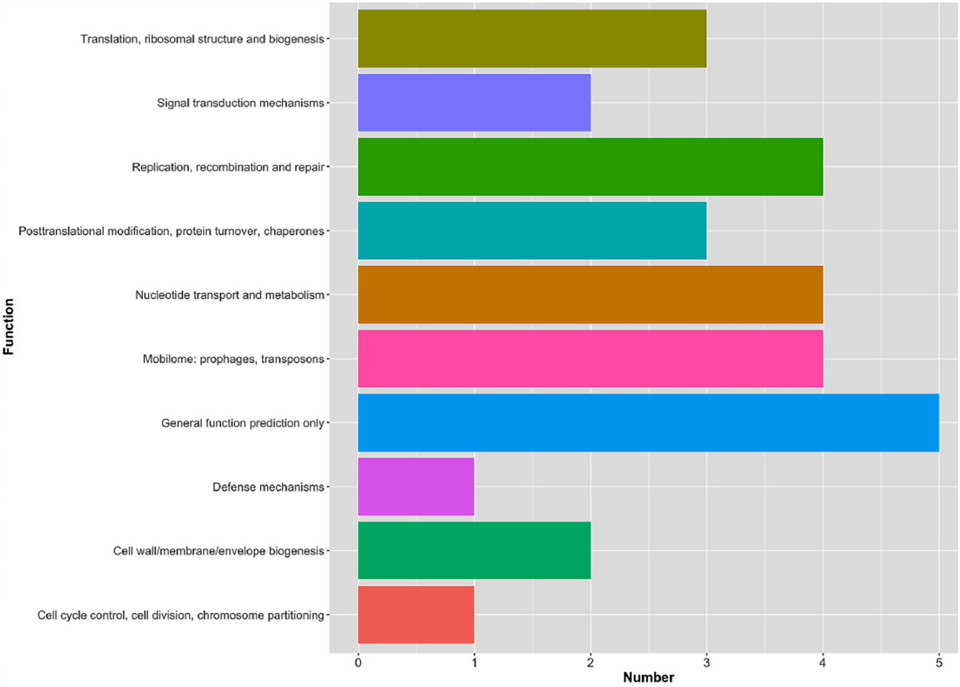

Fig.2. Clusters of Orthologous Groups classification of annotated proteins

Plate IV

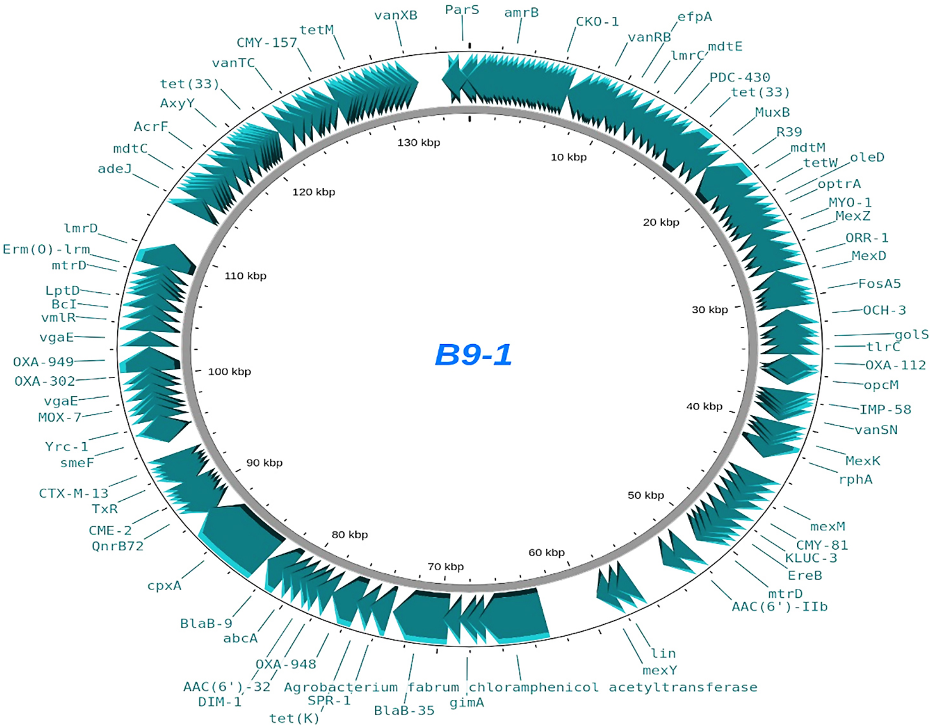

Fig.3. Circular map of genome representing different classes of antibiotics