Virus Genes
DOI 10.1007/s11262-015-1222-9

Isolation and characterization of a bacteriophage with broad host range, displaying potential in preventing bovine diarrhoea

Taruna Anand[1] · Rajesh K. Vaid[1] · Bidhan Ch. Bera[1] · Sanjay Barua[1] · T. Riyesh[1] · N. Virmani[2] · Neeraj Yadav[1] · Praveen Malik[1]

Received: 20 January 2015 / Accepted: 23 June 2015

Abstract Phage therapy has been previously tried for treatment of diarrhoea in calves, pigs and lambs but those trials were conducted without any detailed information of used phages. Here, we report isolation of a broad-spectrum phage which showed bactericidal activity against 47.3 % of calf diarrhoeal isolates of *Escherichia coli*, in vitro. The isolated phage resembled the characteristics of *Myoviridae* family and showed ~97 % similarity with earlier reported bacteriophages of sub family-*Tevenvirinae*, genus-T4-like virus, based on nucleotide sequence of major head protein—gp23 gene. The phage exhibits the potential to be used as drug substitute tool against *E. coli* causing diarrhoea in cattle in farm environments.

Keywords Bacteriophage · Electron microscopy · Myoviridae · Diarrhoea · *E. coli*

Introduction

Calf diarrhoea causes major losses in dairy animal husbandry because of high calf mortality and morbidity. Among the bacterial agents, enterotoxigenic *Escherichia coli* (ETEC) K99+ is implicated quite frequently [1, 2]. Neonatal mortality in large and small ruminants of India, which varies from 12.5 to 30 %, is of major economic importance [3]. Apart from the importance of ETEC from public health aspect, the prevalence of antimicrobial-resistant ETEC and other diarrhoeal *E. coli* strains dissuade use of antimicrobial therapy in diarrhoea [4]. Although K99, F41 bacterin vaccines are available for use in pregnant cattle, matching the right protective antigens with the pathogens present in a farm population is tricky [5]. Bacteriophage (phage) therapy is considered to be an effective alternative for antibiotics [6]. This was widely used in the middle of last century, mainly in East Europe and erstwhile Russia; however, phage therapy fell out of favour due to the advent of antibiotic era.

Phage therapy for treatment of diarrhoea has been tried off and on in bovines [7, 8], pigs, lambs [7] and even in humans [9]. Oral phage treatment in a mouse model of intestinal carriage was able to successfully clear *E. coli* O157:H7 from mice [10]. Given the increased cost of discovery of novel antimicrobials and antimicrobial resistance emergence and emerging promise of bacteriophage therapy, the present study was undertaken to isolate bacteriophage against *E. coli*. Here we report the isolation and characterization of a broad-spectrum bacteriophage showing bactericidal activity against diarrhoeagenic *E. coli* and ETEC isolated from diarrhoeal bovine calves. The isolated bacteriophage could be a potential candidate for phage therapy.

Edited by Joachim Jakob Bugert.

Electronic supplementary material The online version of this article (doi:10.1007/s11262-015-1222-9) contains supplementary material, which is available to authorized users.

✉ Taruna Anand
tarunandri@gmail.com

[1] Veterinary Type Culture Collection, Sirsa Road, Hisar, Haryana, India

[2] National Research Centre on Equines, Sirsa Road, Hisar, India

Published online: 15 July 2015

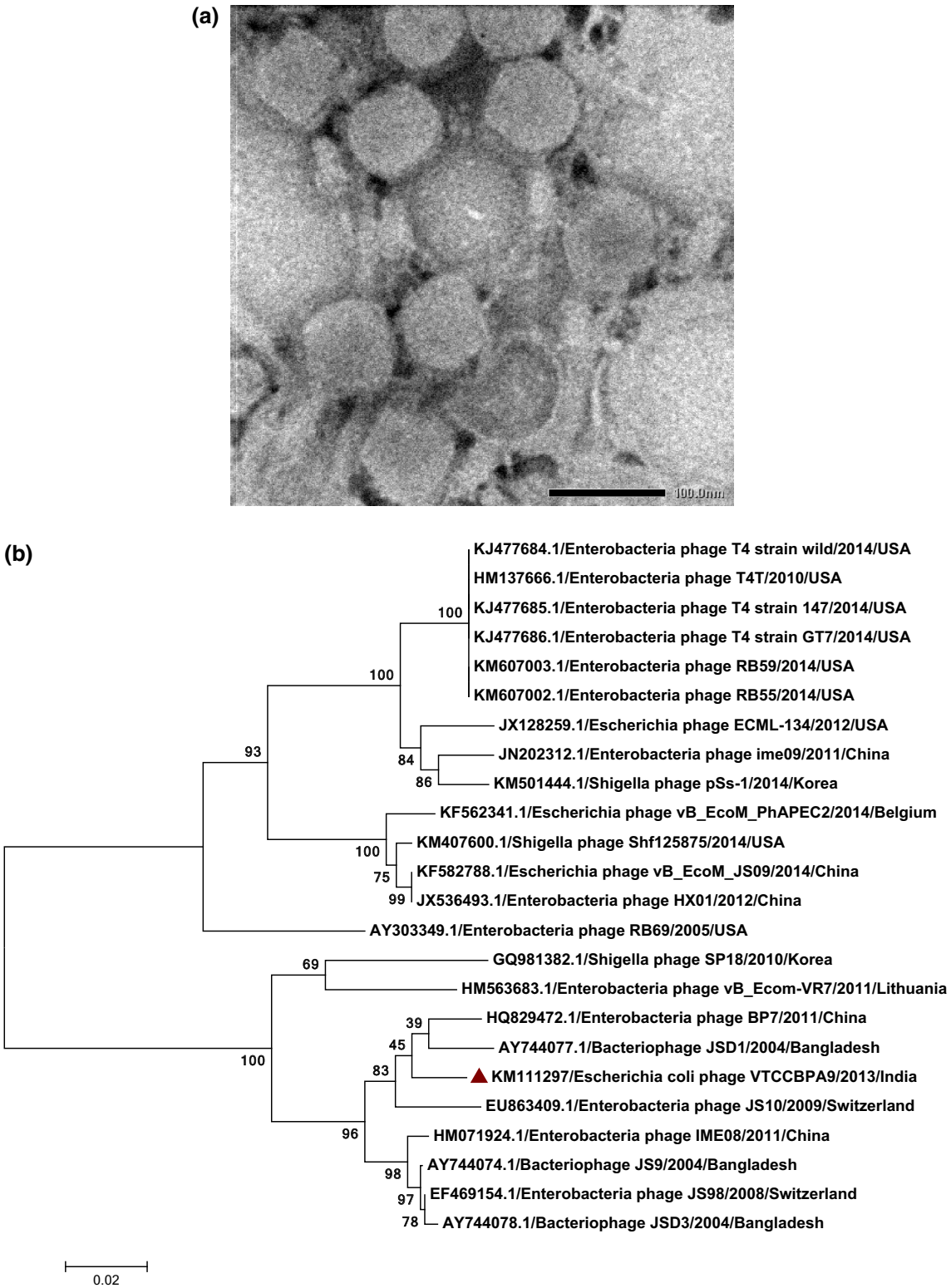

Fig. 1 **a** Typical phage morphology observed under the electron microscope. **b** Phylogenetic analysis of *gp23* gene sequence of phage VTCCBPA9 (neighbour-joining method, boot strap, 1000 replicates)

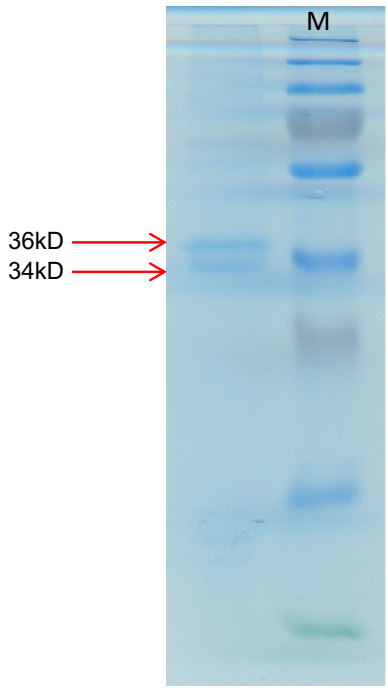

Fig. 2 Protein profile of VTCCBPA9 in 12 % SDS-PAGE. *M*: protein standard

Isolation and characterization of bacteriophage against *E. coli*

An isolate of *E. coli* previously biochemically identified and preserved in the Veterinary Type Culture Collection (VTCC), Hisar, Haryana, India; under the Accession no. VTCCBAA16 was used as a host to enrich bacteriophage from soil samples obtained from a stud farm. One gram of soil was diluted in 40 mL PBS and mixed with 5 mL of 5× nutrient broth (NB); equal amount of grown bacterial culture and shaker incubated at 37 °C, followed by centrifugation at 10,000 rpm for 10 min and filtration through a 0.22-µm PVDF filter. The bacteriophage activity was detected using spot assay by placing 5 µL of filtrate on nutrient agar (NA) seeded with host bacteria. The filtrate was serially diluted and 100 µL of each dilution was plated upon enrichment host by double-agar layer technique [11]. One clearly separated plaque was purified three times by picking 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 presterilized 2 % gelatin) and replating. Plaque characteristics were recorded and phage titre was determined.

For preparation of concentrated phage suspension, 2 mL of exponentially growing host culture was pelleted and suspended in SM buffer. For preparation of crude lysate (CL), phage suspension (10^9 PFU) was added to SM buffer and co-incubated for 20 min at 37 °C, followed by addition of 50 mL NB and shaker incubated for 8–12 h at 37 °C. After that 10 mL chloroform was added to the CL and kept for 10 min without disturbance. CL was now centrifuged at 8300 rpm for 10 min, and pancreatic Dnase I and Rnase to a final concentration of 1 µg/mL were added to the supernatant and incubated for 30 min at room temperature. NaCl and PEG 8000 (Sigma Aldrich) were added to the supernatant to final concentrations of 1 M and 10 % (wt/vol), respectively. After centrifugation and pouring of the supernatant, the bacteriophage pellet was dissolved in SM buffer and re-purified by extraction with chloroform (1:1 v/v) and centrifugation. The isolated bacteriophage preparation was reposited in VTCC with Accession no. VTCCBPA9.

The biological activity of the bacteriophage VTCCBPA9 against ETEC and calf diarrhoeal *E. coli* isolates was tested. 2 µL of the concentrated phage suspension was spotted on the bacterial lawn and incubated overnight at 37 °C. Development of a clear zone after incubation was indicative of bactericidal activity.

Phage DNA extracted using QIAmp DNA mini kit (Qiagen, Bethesda, CA, USA) according to the manufacturer's protocol was used for PCR amplification of the central portion (850 bp) of *gp23* gene encoding for major capsid protein, using Mzia1 (5′-TGTTATIGGTATGG TICGICGTGCTAT-3′) and CAP8 (5′-GAAGTTACCTT CACCACGACCGG-3′) primers [12]. Amplicon was purified using Gel Extraction Kit (Sigma–Aldrich, USA), and the purified products were cloned into pGEM-T Easy vector (M/s Promega Corporation, Madison, WI, USA) as per the manufacturer's protocol. The recombinant plasmid DNAs were verified by colony PCR and restriction enzyme analysis (REA). Three clones were sequenced commercially employing automated DNA sequencer (ABI 3130 Genetic Analyzer). The partial *gp*23 sequence of the isolated phage has been deposited in the GenBank database, NCBI under Accession no. KM111297.

For phylogenetic analysis, the gene sequences of all the related members were retrieved from the NCBI database and aligned using ClustalW method of molecular evolutionary genetic analysis (MEGA5) program [13]. Phylogenetic tree was constructed using neighbour-joining method in MEGA5 with bootstrap analysis using 1000 replicates.

The protein profile of VTCCBPA9 was analysed by mixing with the sample buffer (62.5 mM Tris HCl, 2 % (w/v) SDS, 10 % (w/v) glycerol, 5 % (v/v) 2-mercaptoethanol, 0.001 % (w/v) bromophenol blue and heating in a boiling water bath for 5 min. Polyacrylamide gel electrophoresis (PAGE) was performed and the gel was stained with Coomassie stain [14]. Structural protein profile of phage was analysed using AlphaEaseFC software (Alpha Innotech).

Table 1 Amino acid changes observed in the central portion of the *gp23* gene

	10	22	24	56	66	67	69	75	76	77	78	81
KM111297/*Escherichia coli* phage VTCCBPA9/2013/India	H	L	N	N	E	S	E	K	V	L	E	K
HQ829472.1/Enterobacteria phage BP7/2011/China	.	.	.	D	K	.	.	.	.	.	.	.
AY303349.1/Enterobacteria phage RB69/2005/USA	N	M	S	D	K	K	P	T	Q	T	K	D
HM071924.1/Enterobacteria phage IME08/2011/China	.	.	.	.	.	T	.	.	.	.	.	.
EF469154.1/Enterobacteria phage JS98/2008/Switzerland	.	.	.	.	.	T	.	.	.	.	.	.
EU863409.1/Enterobacteria phage JS10/2009/Switzerland	.	.	.	.	Q	T	.	.	.	.	.	.
GQ981382.1/Shigella phage SP18/2010/Korea	.	.	.	.	Q	T	.	.	.	.	.	.
HM563683.1/Enterobacteria phage vB_Ecom-VR7/2011/Lithuania	.	.	.	.	.	A	.	.	.	.	T	.
KF582788.1/Escherichia phage vB_EcoM_JS09/2014/China	N	M	S	D	K	K	P	T	T	T	V	D
JX536493.1/Enterobacteria phage HX01/2012/China	N	M	S	D	K	K	P	T	T	T	V	D
KM407600.1/Shigella phage Shf125875/2014/USA	N	M	S	D	K	K	P	T	T	T	V	D
AY744074.1/Bacteriophage JS9/2004/Bangladesh	.	.	.	.	.	T	.	.	.	.	.	.
KF562341.1/Escherichia phage vB_EcoM_PhAPEC2/2014/Belgium	N	M	S	D	K	K	P	T	T	T	V	D
AY744077.1/Bacteriophage JSD1/2004/Bangladesh	–	–	–	D	Q	T	.	.	.	.	.	.
AY744078.1/Bacteriophage JSD3/2004/Bangladesh	–	–	–	.	.	T	.	.	.	.	.	.
JN202312.1/Enterobacteria phage ime09/2011/China	N	M	S	D	K	K	P	T	E	T	T	D
KM607003.1/Enterobacteria phage RB59/2014/USA	N	M	S	D	K	K	P	T	Q	T	T	D
KM607002.1/Enterobacteria phage RB55/2014/USA	N	M	S	D	K	K	P	T	Q	T	T	D
KM501444.1/Shigella phage pSs-1/2014/Korea	N	M	S	D	K	K	T	T	Q	T	T	D
KJ477686.1/Enterobacteria phage T4 strain GT7/2014/USA	N	M	S	D	K	K	P	T	Q	T	T	D
KJ477685.1/Enterobacteria phage T4 strain 147/2014/USA	N	M	S	D	K	K	P	T	Q	T	T	D
KJ477684.1/Enterobacteria phage T4 strain wild/2014/USA	N	M	S	D	K	K	P	T	Q	T	T	D
HM137666.1/Enterobacteria phage T4T/2010/USA	N	M	S	D	K	K	P	T	Q	T	T	D
JX128259.1/Escherichia phage ECML-134/2012/USA	N	M	S	D	K	K	P	T	Q	T	V	D

	84	88	89	92	93	94	95	98	99	100	103	106
KM111297/*Escherichia coli* phage VTCCBPA9/2013/India	S	E	A	A	A	H	F	V	E	A	V	T
HQ829472.1/Enterobacteria phage BP7/2011/China	.	.	.	.	.	.	.	.	.	.	.	A
AY303349.1/Enterobacteria phage RB69/2005/USA	T	Q	E	T	V	Y	L	S	A	Q	I	S
HM071924.1/Enterobacteria phage IME08/2011/China	.	.	.	.	.	.	.	.	.	.	.	G
EF469154.1/Enterobacteria phage JS98/2008/Switzerland	.	.	.	S	.	.	.	.	.	.	.	G
EU863409.1/Enterobacteria phage JS10/2009/Switzerland	.	.	.	.	.	.	.	.	.	.	.	A
GQ981382.1/Shigella phage SP18/2010/Korea	.	.	.	S	.	.	.	.	.	.	.	A
HM563683.1/Enterobacteria phage vB_Ecom-VR7/2011/Lithuania	.	.	.	S	.	.	Y	.	.	.	.	A
KF582788.1/Escherichia phage vB_EcoM_JS09/2014/China	T	Q	E	T	V	Y	L	S	A	I	L	G
JX536493.1/Enterobacteria phage HX01/2012/China	T	Q	E	T	V	Y	L	S	A	I	L	G
KM407600.1/Shigella phage Shf125875/2014/USA	T	Q	E	T	V	Y	L	S	A	I	L	G
AY744074.1/Bacteriophage JS9/2004/Bangladesh	.	.	.	S	.	.	.	.	.	.	.	G
KF562341.1/Escherichia phage vB_EcoM_PhAPEC2/2014/Belgium	T	Q	E	T	V	Y	L	S	A	V	L	S
AY744077.1/Bacteriophage JSD1/2004/Bangladesh	.	.	.	.	.	.	.	.	.	.	.	A
AY744078.1/Bacteriophage JSD3/2004/Bangladesh	.	.	.	S	.	.	.	.	.	.	.	G
JN202312.1/Enterobacteria phage ime09/2011/China	T	Q	D	T	V	Y	L	S	A	Q	I	S
KM607003.1/Enterobacteria phage RB59/2014/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G
KM607002.1/Enterobacteria phage RB55/2014/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G
KM501444.1/Shigella phage pSs-1/2014/Korea	T	Q	D	T	V	Y	L	S	A	Q	I	.
KJ477686.1/Enterobacteria phage T4 strain GT7/2014/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G
KJ477685.1/Enterobacteria phage T4 strain 147/2014/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G
KJ477684.1/Enterobacteria phage T4 strain wild/2014/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G

Table 1 continued

	84	88	89	92	93	94	95	98	99	100	103	106
HM137666.1/Enterobacteria phage T4T/2010/USA	T	Q	E	T	V	Y	L	S	V	Q	I	G
JX128259.1/Escherichia phage ECML-134/2012/USA	T	Q	E	T	V	Y	L	S	A	V	I	S

	116	118	119	120	121	125	127	129	174	212	228	230
KM111297/*Escherichia coli* phage VTCCBPA9/2013/India	A	T	A	L	I	K	A	L	S	I	N	V
HQ829472.1/Enterobacteria phage BP7/2011/China	.	.	.	.	.	.	.	.	.	.	.	.
AY303349.1/Enterobacteria phage RB69/2005/USA	E	I	K	Q	M	A	V	I	A	V	.	.
HM071924.1/Enterobacteria phage IME08/2011/China	.	.	.	.	V	Q	.	I	.	.	.	.
EF469154.1/Enterobacteria phage JS98/2008/Switzerland	.	.	.	.	V	Q	.	I	.	.	.	.
EU863409.1/Enterobacteria phage JS10/2009/Switzerland	.	.	.	.	L	Q	.	.	.	.	.	.
GQ981382.1/Shigella phage SP18/2010/Korea	.	.	.	.	L	.	.	.	.	.	.	.
HM563683.1/Enterobacteria phage vB_Ecom-VR7/2011/Lithuania	E	.	K	.	L	.	.	I	.	.	.	.
KF582788.1/Escherichia phage vB_EcoM_JS09/2014/China	E	K	K	Q	M	A	V	I	A	V	L	.
JX536493.1/Enterobacteria phage HX01/2012/China	E	K	K	Q	M	A	V	I	A	V	L	.
KM407600.1/Shigella phage Shf125875/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	.
AY744074.1/Bacteriophage JS9/2004/Bangladesh	.	.	.	.	V	Q	.	I	.	.	.	.
KF562341.1/Escherichia phage vB_EcoM_PhAPEC2/2014/Belgium	E	K	K	Q	M	A	V	I	A	V	L	P
AY744077.1/Bacteriophage JSD1/2004/Bangladesh	.	.	.	.	.	.	.	.	.	.	.	.
AY744078.1/Bacteriophage JSD3/2004/Bangladesh	.	.	.	.	V	Q	.	I	.	.	.	.
JN202312.1/Enterobacteria phage ime09/2011/China	E	K	K	Q	M	A	V	I	A	V	L	P
KM607003.1/Enterobacteria phage RB59/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	P
KM607002.1/Enterobacteria phage RB55/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	P
KM501444.1/Shigella phage pSs-1/2014/Korea	E	K	K	Q	M	A	V	I	A	V	L	P
KJ477686.1/Enterobacteria phage T4 strain GT7/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	P
KJ477685.1/Enterobacteria phage T4 strain 147/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	P
KJ477684.1/Enterobacteria phage T4 strain wild/2014/USA	E	K	K	Q	M	A	V	I	A	V	L	P
HM137666.1/Enterobacteria phage T4T/2010/USA	E	K	K	Q	M	A	V	I	A	V	L	P
JX128259.1/Escherichia phage ECML-134/2012/USA	E	K	K	Q	M	V	V	I	A	V	L	P

	232	281	282	283	284
KM111297/*Escherichia coli* phage VTCCBPA9/2013/India	A	K	S	D	P
HQ829472.1/Enterobacteria phage BP7/2011/China	.	I	I	A	S
AY303349.1/Enterobacteria phage RB69/2005/USA	S	–	–	–	–
HM071924.1/Enterobacteria phage IME08/2011/China	.	I	I	A	S
EF469154.1/Enterobacteria phage JS98/2008/Switzerland	.	I	I	A	S
EU863409.1/Enterobacteria phage JS10/2009/Switzerland	.	I	I	A	S
GQ981382.1/Shigella phage SP18/2010/Korea	.	I	I	A	S
HM563683.1/Enterobacteria phage vB_Ecom-VR7/2011/Lithuania	.	I	I	A	S
KF582788.1/Escherichia phage vB_EcoM_JS09/2014/China	S	I	I	A	S
JX536493.1/Enterobacteria phage HX01/2012/China	S	I	I	A	S
KM407600.1/Shigella phage Shf125875/2014/USA	S	I	I	A	S
AY744074.1/Bacteriophage JS9/2004/Bangladesh	.	–	–	–	–
KF562341.1/Escherichia phage vB_EcoM_PhAPEC2/2014/Belgium	S	I	I	A	S
AY744077.1/Bacteriophage JSD1/2004/Bangladesh	.	–	–	–	–
AY744078.1/Bacteriophage JSD3/2004/Bangladesh	.	–	–	–	–
JN202312.1/Enterobacteria phage ime09/2011/China	S	I	I	A	S
KM607003.1/Enterobacteria phage RB59/2014/USA	S	I	I	A	S
KM607002.1/Enterobacteria phage RB55/2014/USA	S	I	I	A	S
KM501444.1/Shigella phage pSs-1/2014/Korea	S	I	I	A	S

Table 1 continued

	232	281	282	283	284
KJ477686.1/Enterobacteria phage T4 strain GT7/2014/USA	S	I	I	A	S
KJ477685.1/Enterobacteria phage T4 strain 147/2014/USA	S	I	I	A	S
KJ477684.1/Enterobacteria phage T4 strain wild/2014/USA	S	I	I	A	S
HM137666.1/Enterobacteria phage T4T/2010/USA	S	I	I	A	S
JX128259.1/Escherichia phage ECML-134/2012/USA	S	I	I	A	S

The end dot signifies similarity with KM111297/Escherichia coli phage VTCCBPA9/2013/India at a particular location in the gene sequence

Electron microscopy was done by placing phage solution on formvar/carbon coated grids which were allowed to stand for 5 min for bacteriophage to bind. The grids were blotted with Whatman filter paper and negatively stained with 1 % phosphotungstic acid, pH 7 for 10 s and then washed with 1 drop of deionized water. Electron micrographs were taken on JEOL (Welwyn Garden City, England) electron microscope operating at 80 kV.

Results and discussion

In the present study, a bacteriophage (VTCCBPA9) which formed plaques of <1 mm diameter was isolated and obtained in high titres (3×10^{12} pfu/mL). Electron microscopy of the isolated phage showed morphological similarity to *Myoviridae* family [15] with icosahedral head and a contractile tail with a base plate (Fig. 1a). The dimensions were observed to be—capsid: 86 nm; tail: 100 nm × 20 nm and base plate: 33 nm × 17 nm. Structural protein profile of the isolated phage revealed the presence of two characteristic proteins of ~34 and 36 kD which might correspond to major capsid and tail proteins and few other protein bands of high molecular weight (Fig. 2).

The partial region of the conserved *gp23* gene, routinely used for the characterization of bacteriophages, was cloned and sequenced [12]. The open reading frame (ORF) of *gp23* gene of bacteriophage comprises 1159 bp encoding 519 amino acid (aa) residues for major head protein. The central portion (280–1131 bp) of 851 bp of this gene of isolated phage was amplified using reported primers [12]. Homology analysis of the nucleotide sequence revealed that *gp23* gene of the phage isolate shared ~97 % similarity with reported bacteriophages. Comparative analysis of aa residues of deduced protein revealed six consensus aa substitutions (T/A67S, A/G106T, I281K, I282S, A283D and S284P) among closely related phages (Table 1). However, several consensus aa changes were found in other related Enterobacteria phages. The last four consensus aa substitutions were present in the universally conserved 70 aa sequence between residues 230 and 300 of the T4 capsid gene (12). The gp23 protein play major role in head morphogenesis and variation in central region of this gene has been implicated in interactions between the subunits of gp23 proteins as well as with various head accessory proteins on the surface of the capsid which could result in variation in virion head size and shape (12). The unique six aa substitutions observed in Indian isolate might have some role in head morphogenesis and also support the strong divergence and mosaic designing of this gene. Phylogenetic tree was constructed to find the evolutionary relationship of Indian isolate with other related phage isolates. The topology of the phylogram (Fig. 1b) divided phages into two separate clusters supported by high bootstrap value (93–100 %). The isolates mostly from USA formed one group and other group contained four isolates from Asian region including current isolate from India, and two isolates from Europe. The present isolate was closest to Enterobacteria phage BP7 and Bacteriophage JSD1 isolates—both are T4 type phages of *E. coli* belonging to sub family-*Tevenvirinae* and genus-T4-like virus. Enterobacteria phage BP7 has been reported to show a wide host range among pathogenic *E. coli* strains (46 %) [16]; it has an elongated icosahedral head and a long tail, whereas the VTCCBPA9 phage has almost isometric head and a comparatively short tail. Our isolate showed 97 % similarity with reported phages and it might be either a morphological variant or mutant or an evolved phage belonging to the same family.

Phages of the family *Myoviridae* have been successfully used in phage therapy of various diseases [17–19]. We employed VTCCBPA9 phage to test lysis of ETEC and diarrhoeal isolates previously obtained from cattle and buffalo calves from different states of India and preserved in VTCC. VTCCBPA9 showed bactericidal activity against 47.3 % (62/131) *E. coli* isolates, including 3 ETEC strains. The wide host range of phage suggests its polyvalent nature and a high potential for use against calf-diarrhoea. Thus we isolated a phage with broad activity and demonstrated its biological activity with a potential to be used as drug substitute tool against pathogenic *E. coli,* causing diarrhoea in cattle and buffalo in farm environments.

Acknowledgments This work was supported by Indian Council of Agricultural Research, Department of Agricultural Research and Education (DARE), Ministry of Agriculture, Government of India under the institutional project Grant No. IXX10698. Electron microscopy was carried out at High Security Animal Disease Laboratory, TEM unit, IVRI, Bhopal, M.P., India. I sincerely acknowledge the encouragement and valuable suggestions from Dr. Raj Kumar Singh (Director IVRI, Izatnagar).

References

1. D.J. Reynolds, J.H. Morgan, N. Chanter, P.W. Jones, J.C. Bridger, T.G. Debney, K.J. Bunch, Vet. Rec. **119**, 34–39 (1986)
2. D.R. Snodgrass, H.R. Terzolo, D. Sherwood, I. Campbell, J.D. Menzies, B.A. Synge, Vet. Rec. **119**, 31–34 (1986)
3. G.S. Verma, D.K. Sadana, S.B. Basul, B.A. Sharma, Indian J. Anim. Sci. **50**, 87–90 (1980)
4. N.T. Do, H.P. Cu, N.N. Nguyen, X.H. Nguyen, X.T. Au, T.H. Van, N.Q. Vu, D.J. Trott, Ann. N. Y. Acad. Sci. **1081**, 543–545 (2006)
5. B. Nagy, P.Z. Fekete, Int. J. Med. Microbiol. **295**, 443–454 (2005)
6. W.C. Summers, Annu. Rev. Microbiol. **55**, 437–451 (2001)
7. H.W. Smith, M.B. Huggins, K.M. Shaw, J. Gen. Microbiol. **133**, 1111–1126 (1987)
8. H.W. Smith, M.B. Huggins, J. Gen. Microbiol. **129**, 2659–2675 (1983)
9. S.A. Sarker, S. Mc Callin, C. Barretto, B. Berger, A.C. Pittet, S. Sultana, L. Krause, S. Huq, R. Bibiloni, A. Bruttin, G. Reuteler, H. Brüssow, Virology **434**, 222–232 (2012)
10. H. Sheng, H.J. Knecht, I.T. Kudva, C.J. Hovde, Appl. Environ. Microbiol. **72**, 5359–5366 (2006)
11. M.H. Adams, *Bacteriophages* (Interscience Publishers, New York, 1959), p. 27
12. F. Tetart, C. Desplats, M. Kutateladze, C. Monod, H.W. Ackermann, H.M. Krisch, J. Bacteriol. **183**, 358–366 (2001)
13. K. Tamura, D. Peterson, N. Peterson, G. Stechter, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
14. U.K. Laemmili, Nature **227**, 680–685 (1970)
15. H.W. Ackermann, H.M. Krisch, Arch. Virol. **142**, 2329–2345 (1997)
16. C. Zhang, W. Li, W. Liu, L. Zou, C. Yan, K. Lu, H. Ren, Appl. Environ. Microbiol. **79**, 5559–5565 (2013)
17. G. Bourdin, B. Schmitt, L. Marvin Guy, J.E. Germond, S. Zuber, L. Michot, G. Reuteler, H. Brüssow, Appl. Environ. Microbiol. **80**, 1469–1476 (2014)
18. J.J. Mendes, C. Leandro, C. Mottola, R. Barbosa, F.A. Silva, M. Oliveira, C.L. Vilela, J. Melo-Cristino, A. Górski, M. Pimentel, C. São-José, P. Cavaco-Silva, M. Garcia, J. Med. Microbiol. **63**, 1055–1065 (2014)
19. L. Zhang, H. Bao, C. Wei, H. Zhang, Y. Zhou, R. Wang, Virus Genes **50**, 111–117 (2014)