

Isolation and characterization of lytic bacteriophages against bacteria of veterinary importance

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**Veterinary Type Culture Collection,
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Phages have been preserved worldwide

Name of culture collection

✓ American Type Culture Collection (ATCC), USA

✓ National Biological Resource Center (NBRC), Japan

✓ Laboratoire de Microbiologie et Génétique Moléculaires, France- largest collection of T4 superfamily of bacteriophages

✓ Felix d'Herelle Reference Center for bacterial viruses, Canada

✓ Deutsche Sammlung von Mikroorganismen und Zellkulturen – DSMZ – (German Collection of Microorganisms and Cell Cultures)

✓ The Netherlands Culture Collection of Bacteria (NCCB)

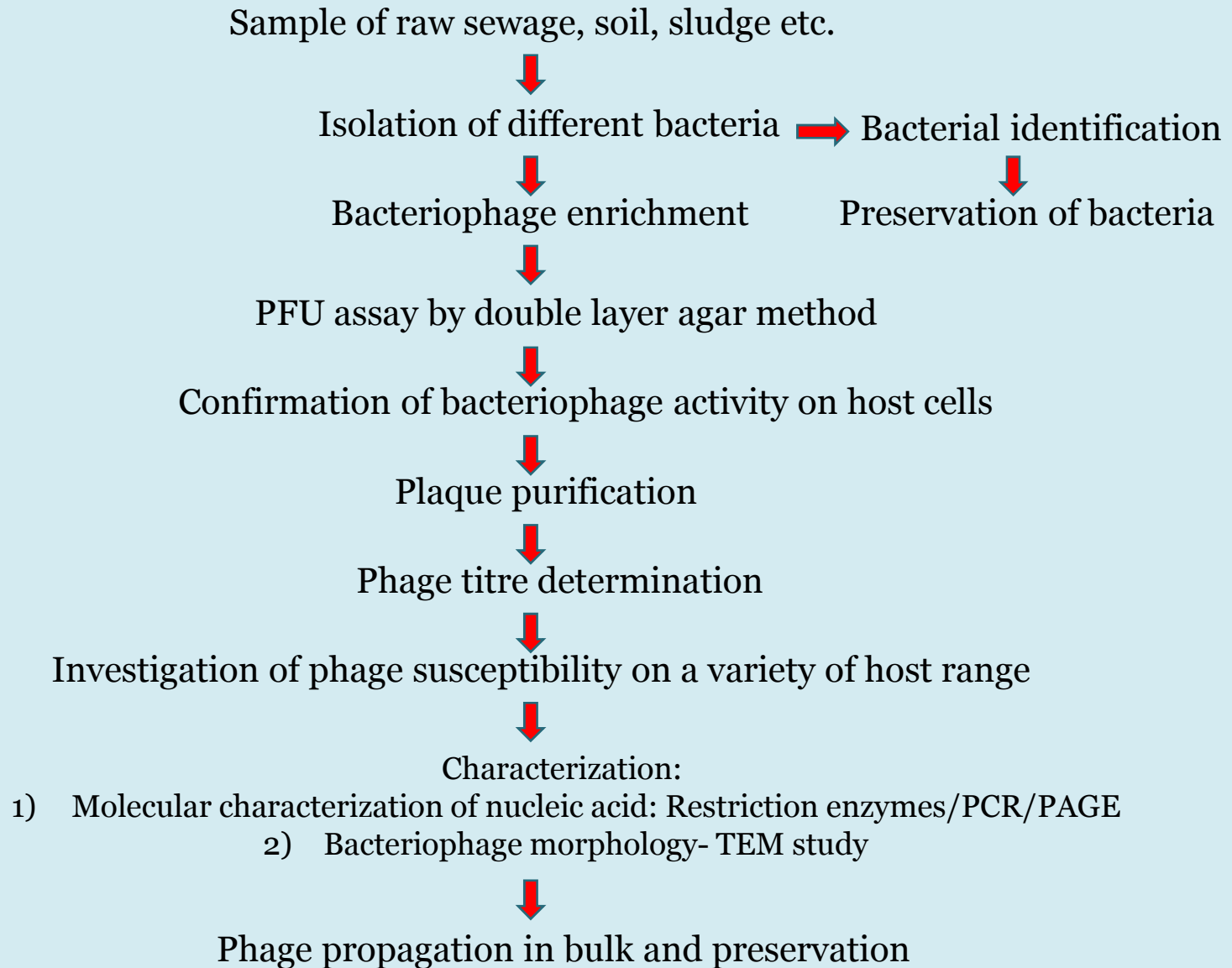
✓ National Collections of Industrial, Food and Marine Bacteria, NCIMB, Scotland

✓ Korea National Research Resource Center- Bacteriophage Bank

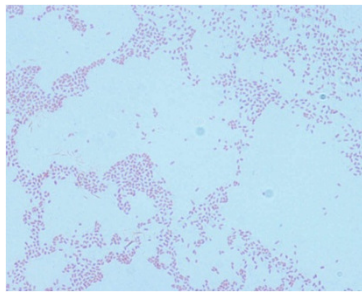
✓ China Center For Virus Culture Collection

Objectives

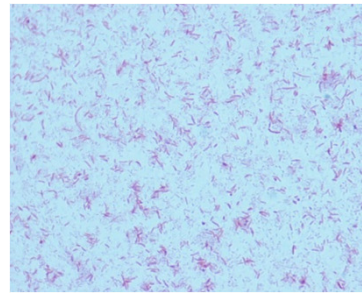
- **To isolate and characterize bacteriophages of different bacteria.**
- **To develop repository of bacteriophages.**



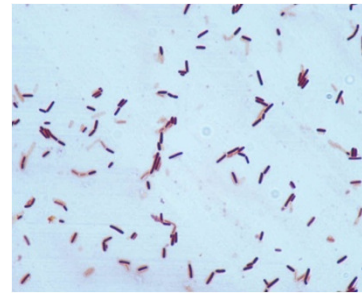
Bacterial host against which bacteriophages isolated



E. coli



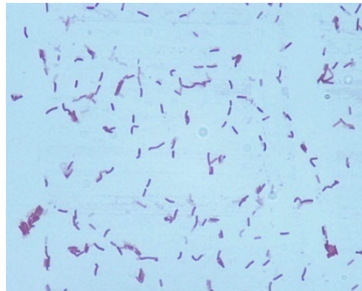
Aeromonas hydrophila



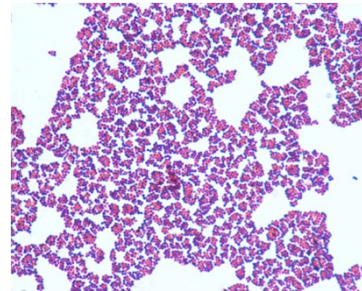
Bacillus subtilis ssp. *subtilis*



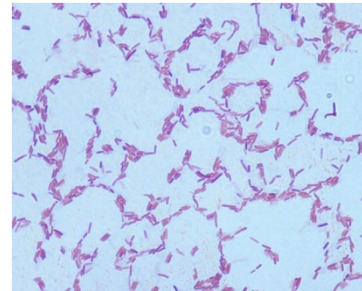
Bacillus sp.



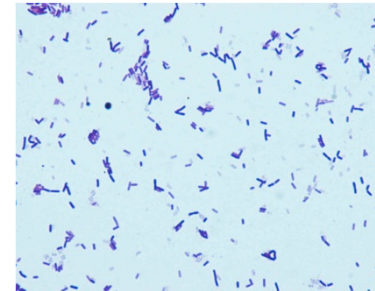
Bacillus sp.



Staphylococcus sciuri

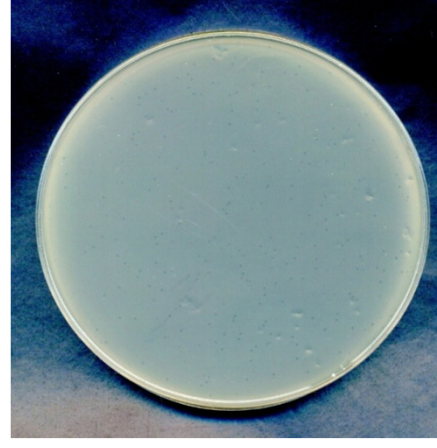
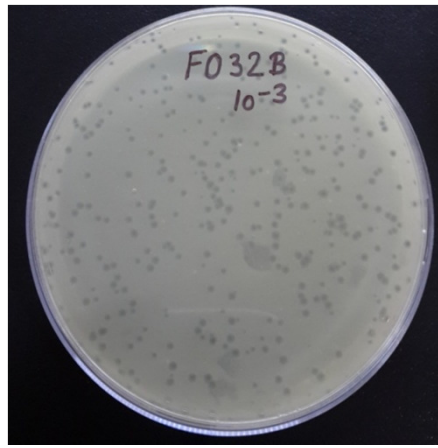
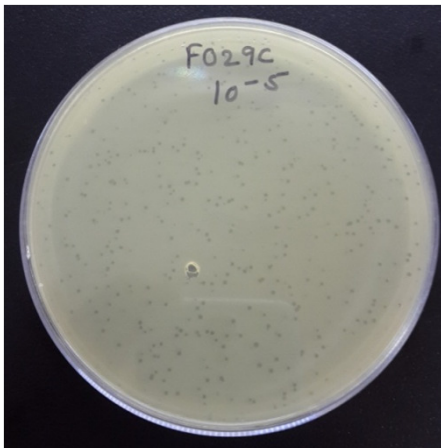
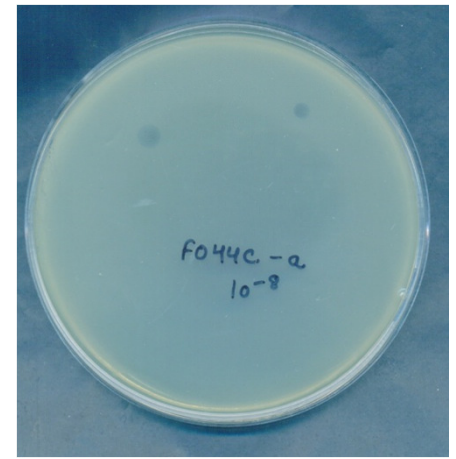
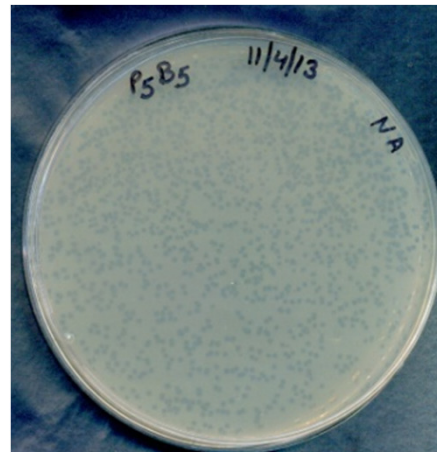
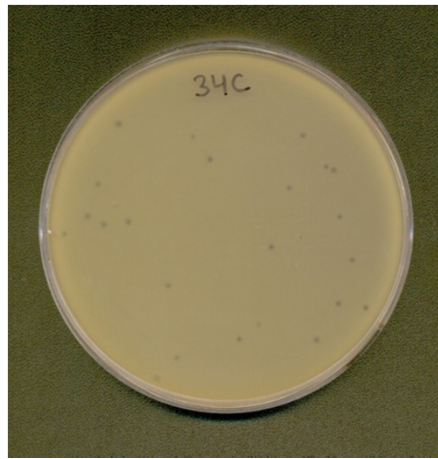
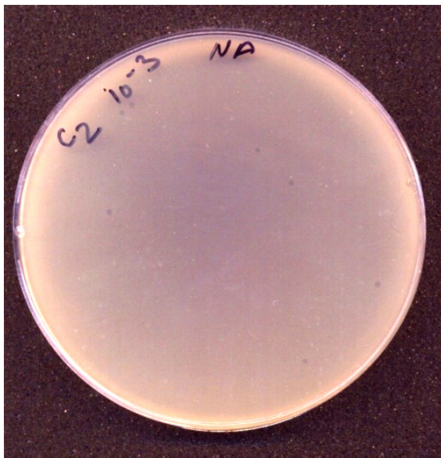


Bacillus spp.



Bacillus spp.

Plaque characteristics





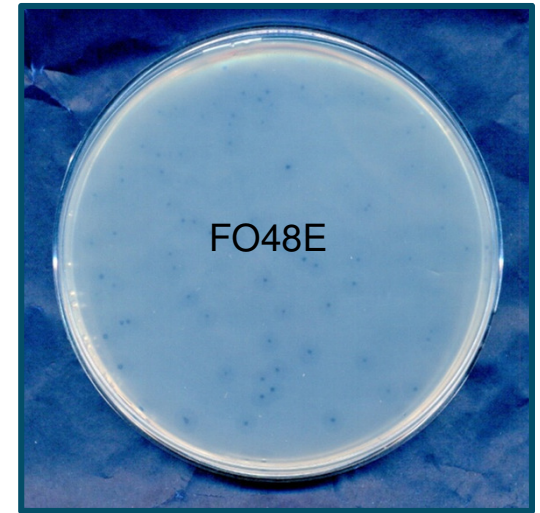
Clear plaques with rough margins,
with a halo zone, 3-4mm dia

Bacillus pumilus



Clear plaques of 1-2mm dia

Pseudomonas mendocina



Clear plaques with a halo zone,
5-6mm dia

Paenibacillus sp.

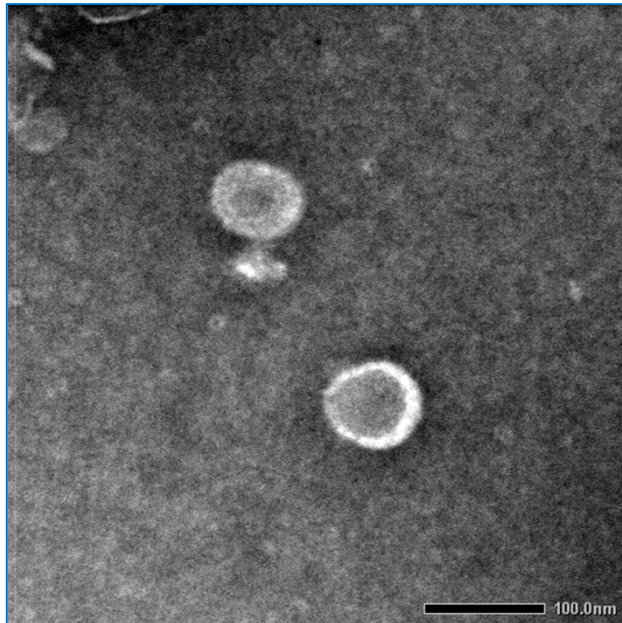
Phages isolated-Plaque characteristics

Bacteria- lab ID	Source of phage	Plaque characteristics	
		Size	Type
Eq37-2	Sewage	1mm	Very clear with smooth margins
Eq16	Soil	<1mm	Very clear in middle with hazy margin, circular
Eq53	Soil	1-2mm	Clear with hazy margins
Eq73-2	Soil	<1mm	Very clear with smooth margins
C1/Fo38A	Soil	1-2mm	Irregular shape, clear
C2/ Fo38B	Soil	3mm	Clear plaques
Eq12B	Soil	2mm	Clear with smooth margins, circular
Fo37	Soil	<1mm,	Very small clear plaques
Fo23c	Sewage	1-2mm	Turbid plaques
Fo34c	Soil	2-3mm	Clear plaques
Fo32B (1)	Soil	2-3mm	Clear plaques with a zone of halo
Fo32B (2)	Soil	2-3mm	Clear plaques
Fo32c	Soil	2-3mm	Clear plaques with hazy margins
Fo35B	Soil	2-3mm	Clear plaques
Fo33A	Soil	6mm,	Turbid plaques with a clear central zone of 1-2mm

The phage stocks have been preserved at -80°C

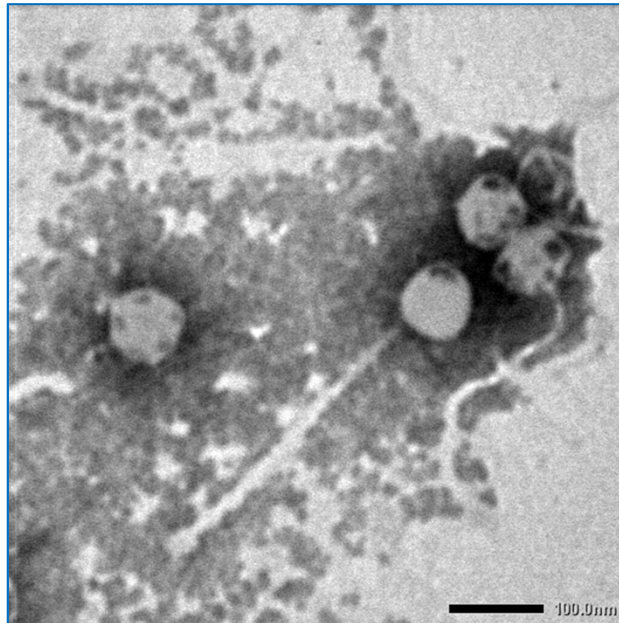
Electron Microscopy

Eq37-2



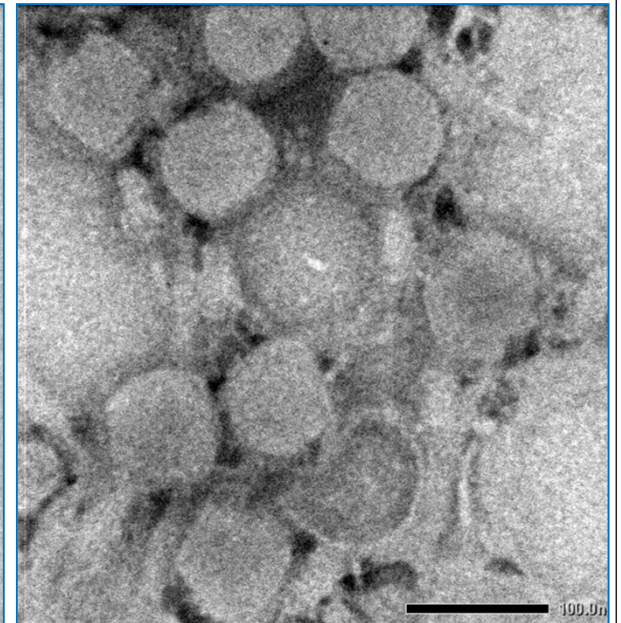
- Capsid dia: 88nm
- Tail length: 5nm
- Base plate width: 45nm
- Tail width: 8nm
- Podoviridae

FO34c



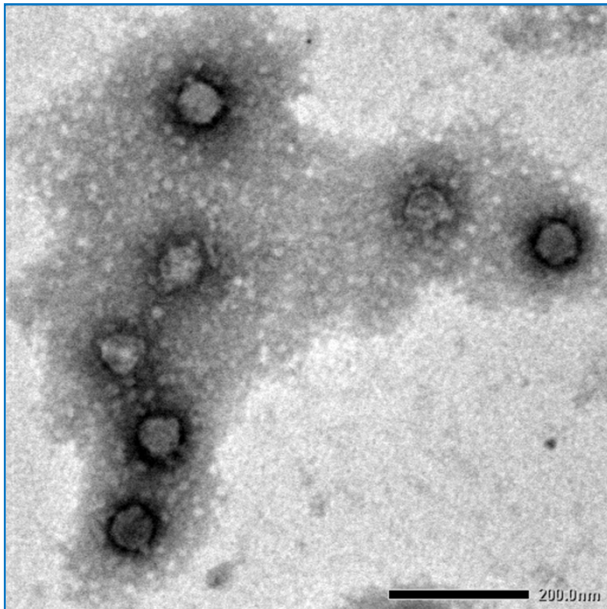
- Capsid dia: 78 nm
- Tail length: 308 nm
- Tail width: 16 nm
- Base Plate width: 24nm
- Base Plate Length: 36 nm
- Siphoviridae

Eq16



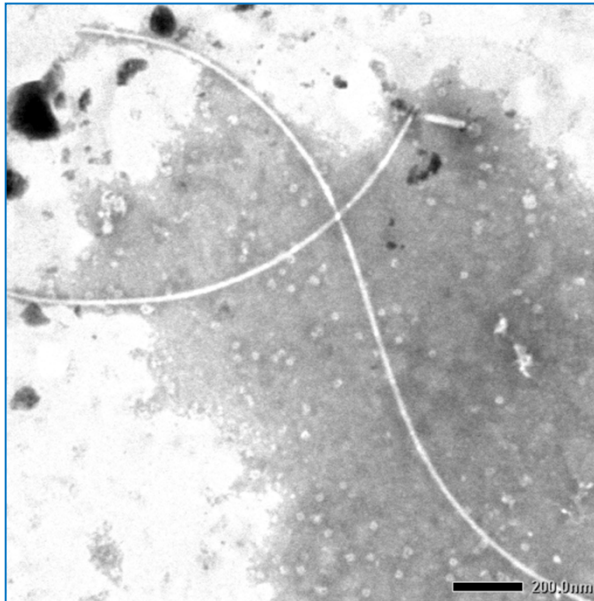
- Capsid dia: 86nm
- Tail length: 100nm
- Tail width: 20nm
- Base Plate width: 33nm
- Base Plate Length: 17nm
- Myoviridae

FO44ca



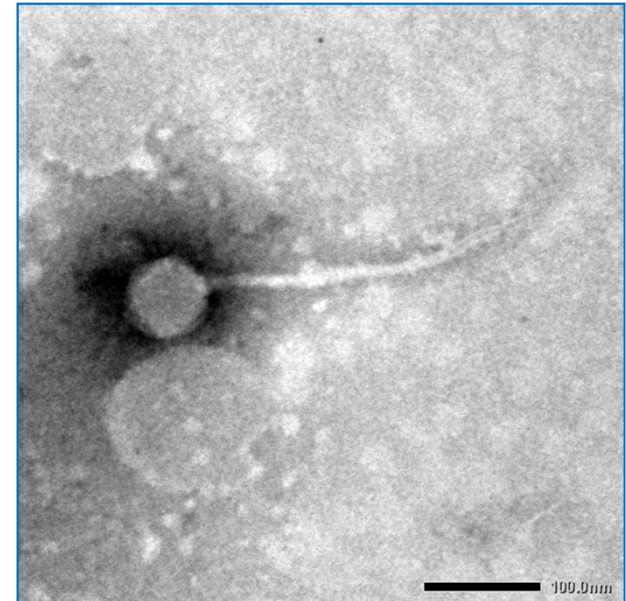
- Capsid dia: 56nm
- Tail length: 5 nm
- Podoviridae

FO44a



- Length: 1.25-2.5um
- Width: 17.7nm
- Inoviridae

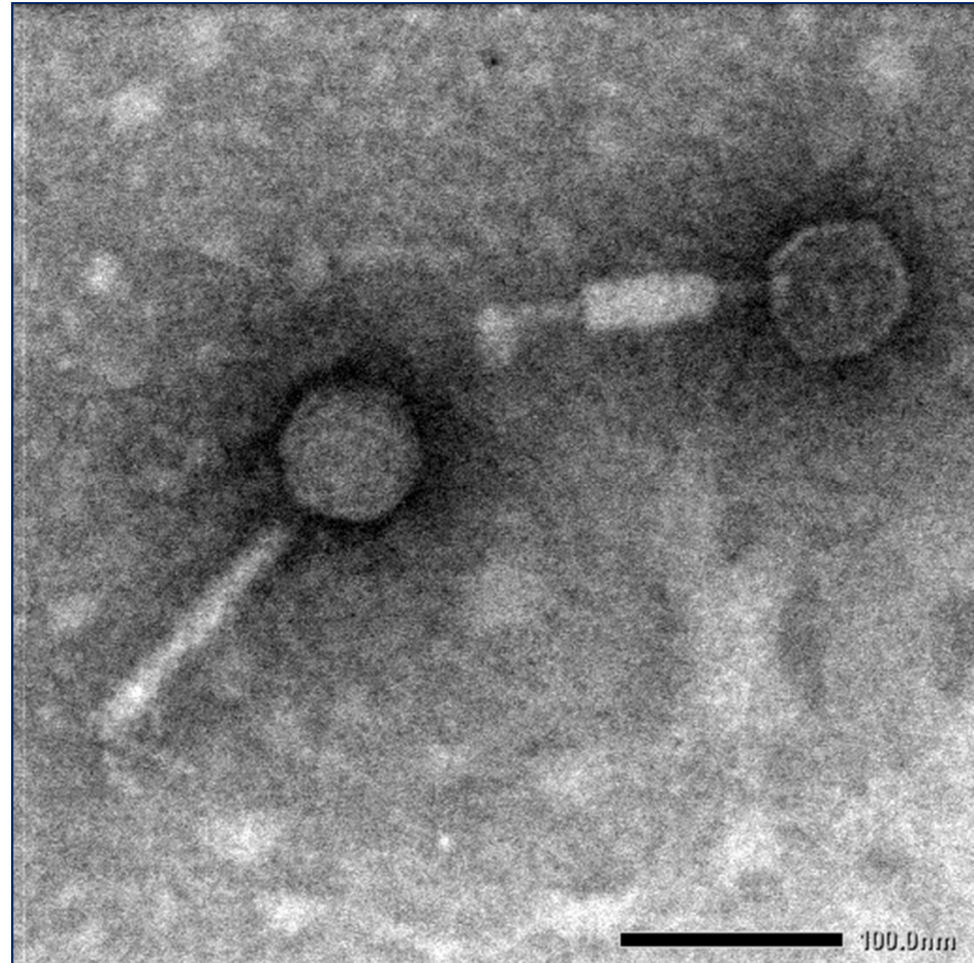
FO35b



- Capsid dia: 69 nm
- Tail length: 265 nm
- Tail width: 10 nm
- Siphoviridae

FO23c-*Aeromonas hydrophila*

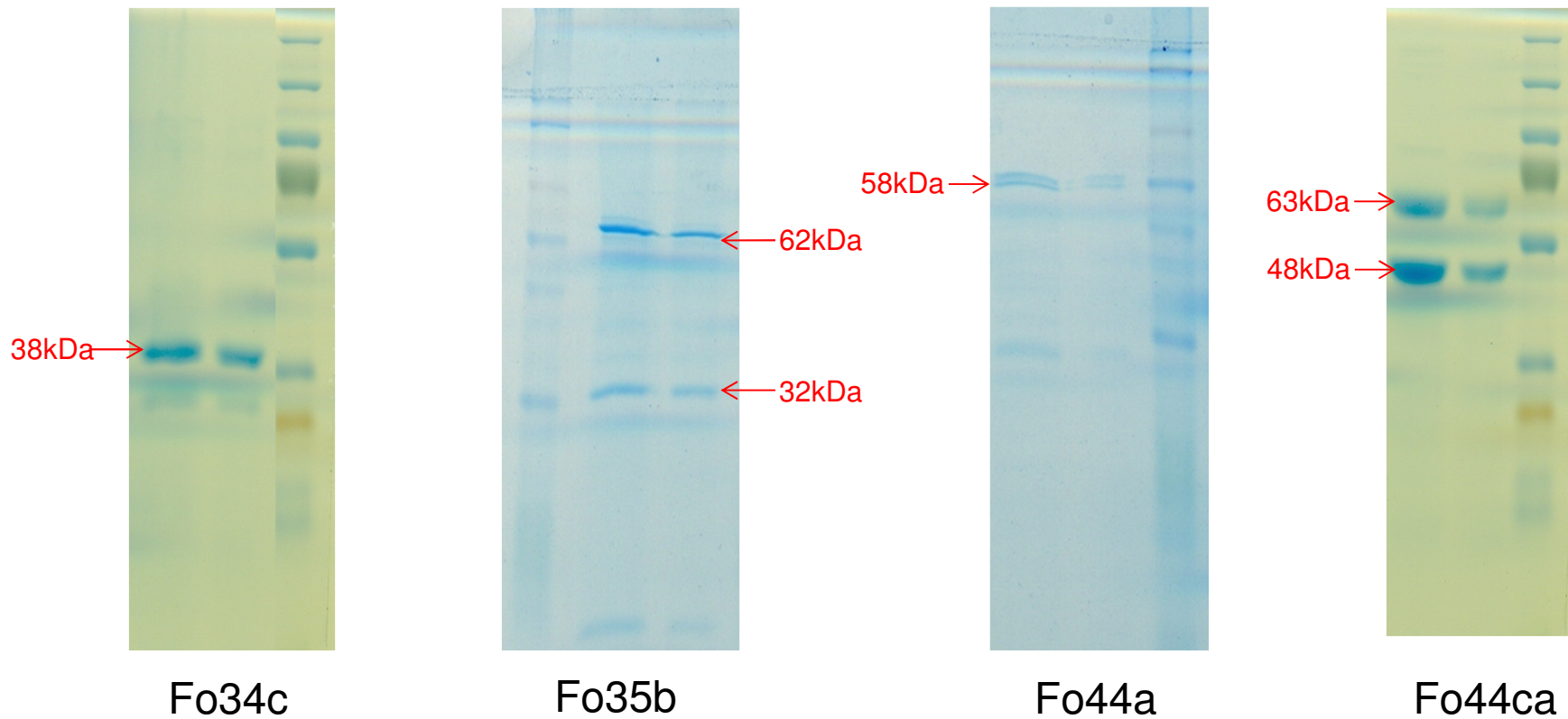
- Capsid dia: 62nm
- Tail length: 138nm
- Tail width: 13nm (contracted)
- Tail width: 19nm (relaxed)
- Base plate
- Myoviridae

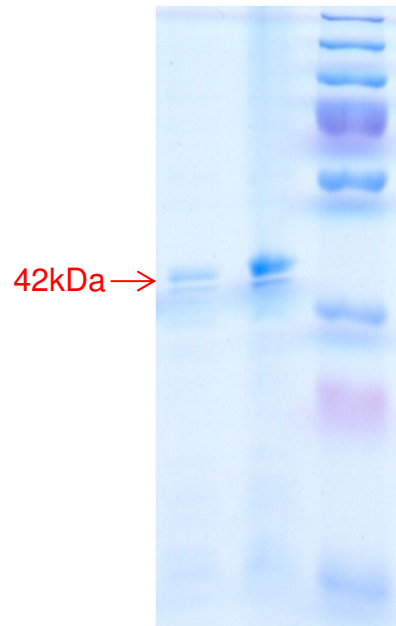


Phage Types

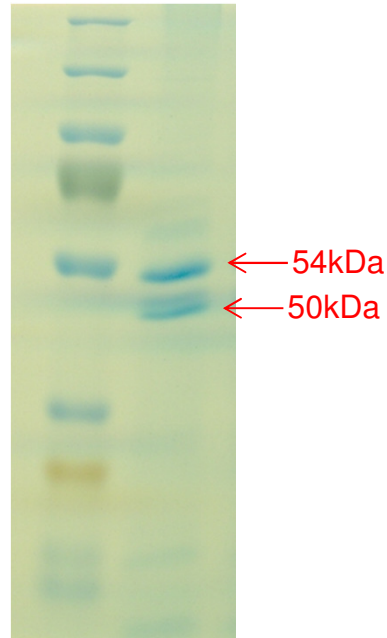
Sample Name	Host	Phage Type/ characteristics observed by TEM
Eq37-2	<i>E. coli</i>	Podoviridae
FO34C	<i>Staphylococcus sciuri</i>	Siphoviridae
FO35B	<i>Bacillus spp.</i>	Siphoviridae
FO44A	<i>Bacillus spp.</i>	Inoviridae
Eq16	<i>E. coli</i>	Myoviridae
FO44Ca	<i>Aeromonas hydrophila</i>	Podoviridae (Novel phage candidate)?
FO23c	<i>Aeromonas hydrophila</i>	Myoviridae

Protein profile of phage isolates

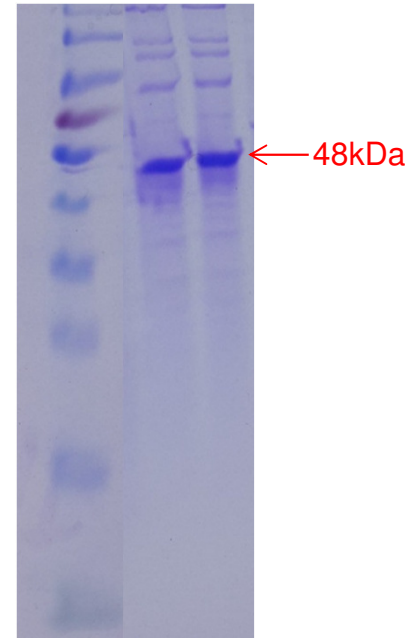




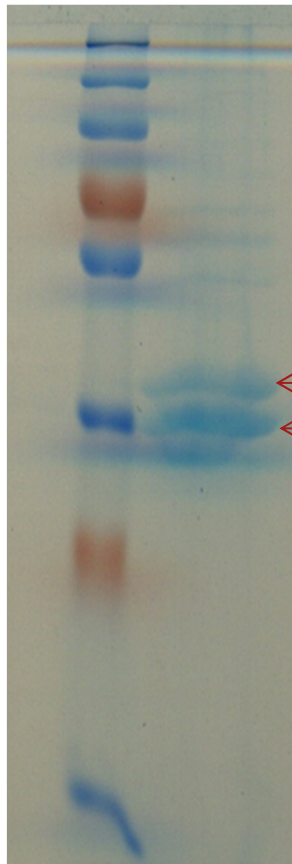
Eeq16



Fo23c

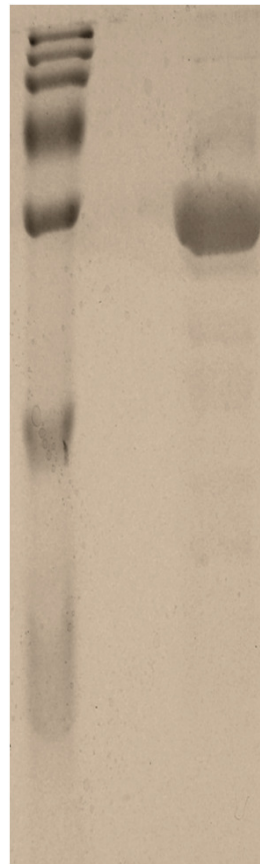


Eeq37-2



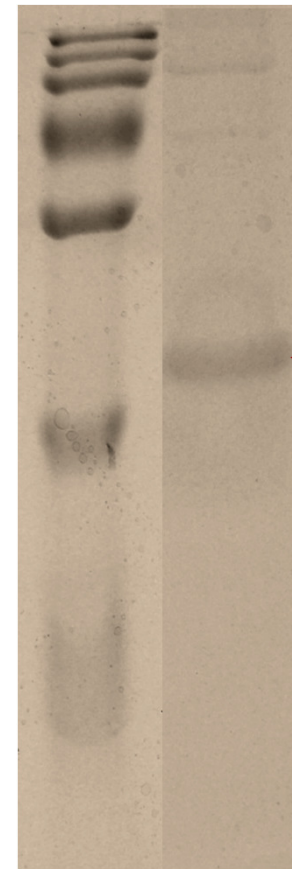
← 38kDa
← 35kDa

Fo48B



← 50kDa

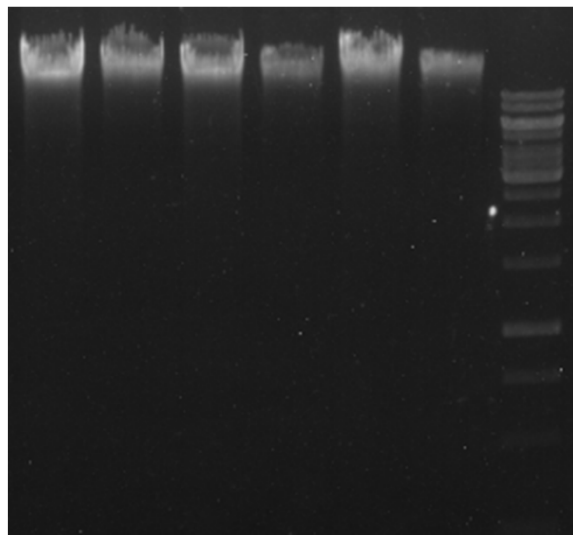
Fo47D



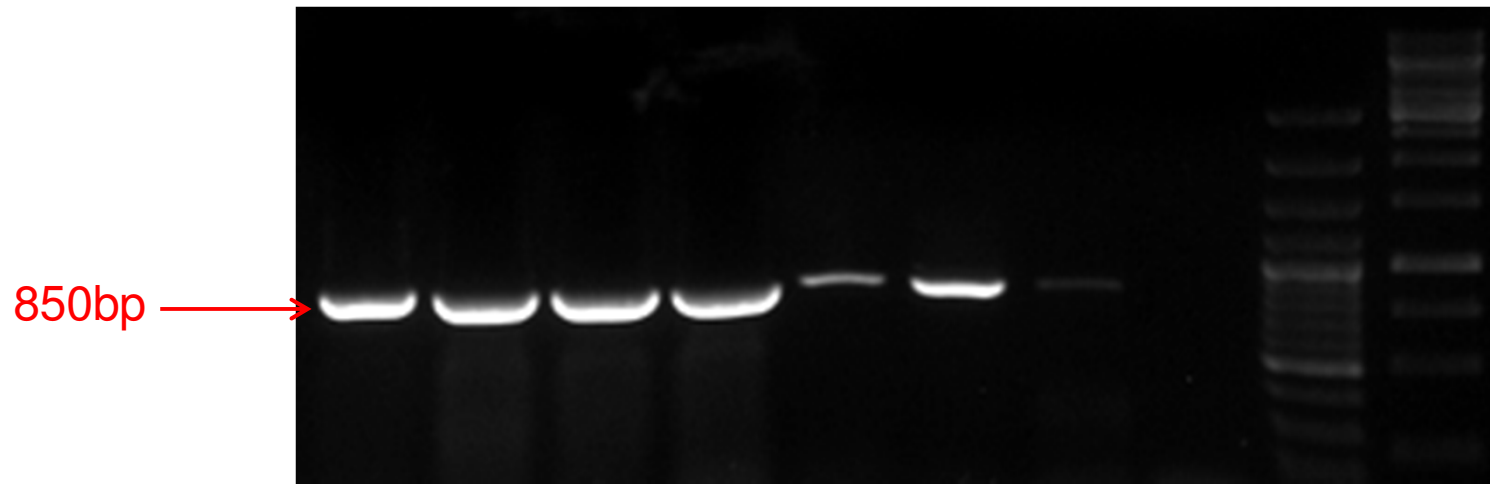
← 40kDa

Fo48E

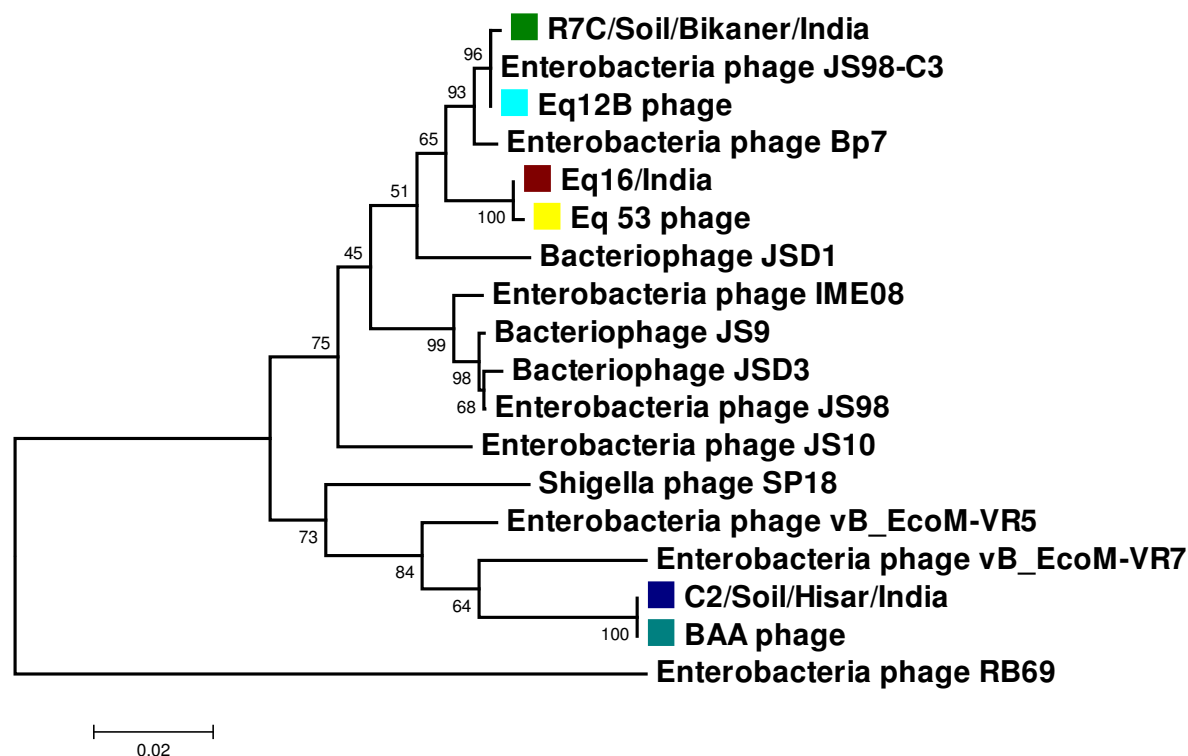
Phage DNA



Phage identification by PCR (gp23 gene)



Phylogenetic analysis



#Kq16/India	KQGVRAIPK	LIATDIDGVQ	PLNPTIGQVT	ALRAVYQKDP	IAAGAKKAFK	PNYAPWANT'S	QGGAAKSTEA	LAASKVLEVG	KIYSEYFAT
#R7C/Soil/Bikaner/IndiaT.S.D...
#C2/Soil/Wazir/India	V.....QT...
#Kq13_phage	V.....QT...
#Kq13_phageK..
#Kq12B_phageS.D...
#Bacteriophage_J59	---?.....?I...
#Bacteriophage_J5D1D...	...QT...	...G...
#Bacteriophage_J5D3?	??.....?I...
#Enterobacteris_phage_Bp7S.D...	...K...
#Enterobacteris_phage_INK08I...
#Enterobacteris_phage_J510QT...	...G...
#Enterobacteris_phage_J595I...
#Enterobacteris_phage_J595-C3	GN.....S.D...
#Enterobacteris_phage_R569N.....K.S.....D...	...KK.P.	...IQIK..	...D...T...QE.
#Enterobacteris_phage_vB_KocX-VR5W...K.....	V.....A...	...I...
#Enterobacteris_phage_vB_KocX-VR7	V.....A...P	...I...
#Shigella_phage_S215	V.....QT...
#Kq16/India	GAAMTQAVIA	VTVDAATADA	AKLDAAVIAL	IEAGKLAELA	KGNATSTAEK	QEGYNGSTON	PNHEKGYRID	KQVIEAKSRQ	LNAYSIKELA
#R7C/Soil/Bikaner/India
#C2/Soil/Wazir/India	..S.....	...G...	...E.K.L...	...I.....
#Kq13_phage	..S.....	...G...	...E.K.L...	...I.....
#Kq13_phage
#Kq12B_phage
#Bacteriophage_J59	..S.....	...G...	V..Q...I.
#Bacteriophage_J5D1A...	...V...Q...
#Bacteriophage_J5D3	..S.....	...KFG...	V..Q...I.
#Enterobacteris_phage_Bp7A...
#Enterobacteris_phage_INK08G...	V..Q...I.
#Enterobacteris_phage_J510A...	...V...	L..Q...Q...
#Enterobacteris_phage_J595	..S.....	...G...	V..Q...I.
#Enterobacteris_phage_J595-C3
#Enterobacteris_phage_R569	..TVYL..SAQ	..ISSS.D..	...KIKQK..	A.V.I.A.....
#Enterobacteris_phage_vB_KocX-VR5	..S.....	...A...	...E.K.L...	...I.....Q...
#Enterobacteris_phage_vB_KocX-VR7	..S.Y.....	...A...	...E.K.L...	...I.....Q...
#Shigella_phage_S215	..S.....	...A...	...L...Q...

#Iq16/India	QDLRAVHMKD	ADAKLSGILA	IKIMLEINRE	VIDWINYSAQ	VGKSGMNTV	GAKAGVYDFQ	DPIDIRGARN	AGESYKALLY	QIDYKAAK IA
#R7C/Soil/Bikaner/India
#C2/Soil/Mysr/India
#S13_phage
#Iq_53_phage
#Iq125_phage
#Bacteriophage_J59
#Bacteriophage_J5D1?
#Bacteriophage_J5D3???
#Interobacteris_phage_Bp7?
#Interobacteris_phage_INK05
#Interobacteris_phage_J510
#Interobacteris_phage_J595
#Interobacteris_phage_J595-C3
#Interobacteris_phage_RB69
#Interobacteris_phage_vB_KcoK-VR5
#Interobacteris_phage_vB_KcoK-VR7
#Shigella_phage_S915
#Iq16/India	RQIGRGKMT	KSDP							
#R7C/Soil/Bikaner/India
#C2/Soil/Mysr/India
#S13_phage
#Iq_53_phage
#Iq125_phage
#Bacteriophage_J59
#Bacteriophage_J5D1
#Bacteriophage_J5D3
#Interobacteris_phage_Bp7
#Interobacteris_phage_INK05
#Interobacteris_phage_J510
#Interobacteris_phage_J595
#Interobacteris_phage_J595-C3
#Interobacteris_phage_RB69
#Interobacteris_phage_vB_KcoK-VR5
#Interobacteris_phage_vB_KcoK-VR7
#Shigella_phage_S915

(Group) ds DNA

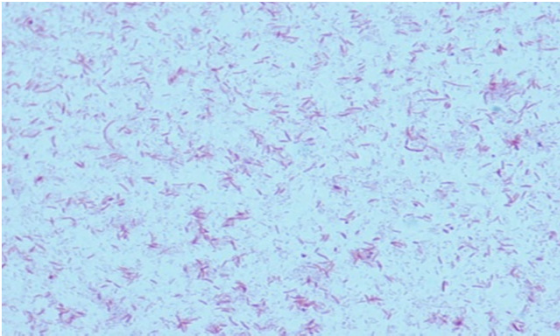
(Order) Caudovirales

(Family) Myoviridae

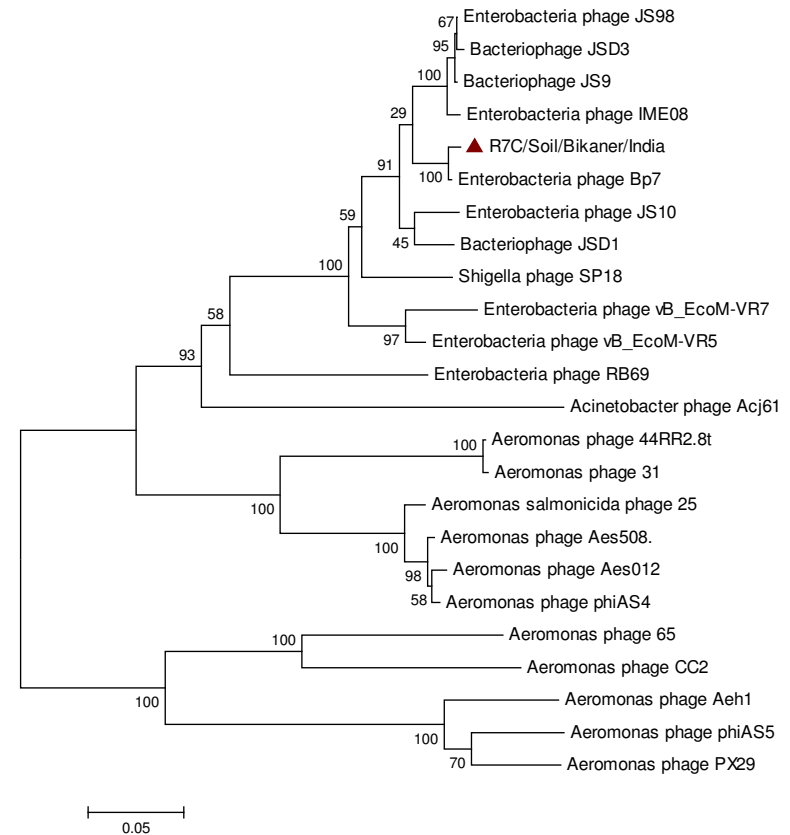
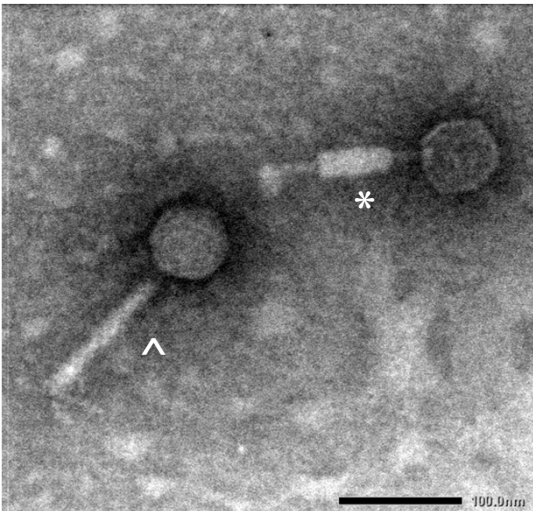
(Sub Family) Tenevirinae

(Genus) T4-like virus

Aeromonas hydrophila phage



PCR detection of (A) aerolysin gene product (252bp) and (B) lip gene product (760bp).



Biological activity against diarrhoeal isolates of *E. coli* analysed by Spot Test

Phage isolate	Accession no. of test host bacteria	Activity
Eq16 (4/9)	LLR001B1	+++
	LLR002A2	+++
	LLR003B1	+
	SDF007A1	+
Eq37-2 (1/9)	LLR002A2	+++



Conclusions

- Diverse kind of bacteriophages were isolated from sewage and soil samples of animal farms.
- Phages varied in plaque characteristics, morphology and protein profiles.
- Electron microscopic analysis classified phages mainly into caudovirales-Myoviridae, Podoviridae and Siphoviridae families.
- Few phages showed biological activity against *E. coli* isolated from calf diarrhoea.....potential application in phage therapy.

Thankyou.....