Table 1. Bacterial strains, bacteriophages and plasmids used in this study.

Bacterium strain	Characteristics	Source or reference
Klebsiella pneumoniae 10693	No plasmid, no drug resistance phage host	American Type Culture Collection (ATCC) 23357
Klebsiella pneumoniae 25 strains	Extended-spectrum β-lactamases (ESBL) strains	林雅嵐, 2001
Enterobacter cloacae 24 strains	ESBL strains	This laboratory
Serratia marcescens 29 strains	Cefotaxime resistant	This laboratory
Escherichia coli 13 strains	G (-) pathogen	This laboratory
Klebsiella oxytoca 1 strains	Cefotaxime resistant	This laboratory
Enterobacter agglomerans 1 strains	ESBL strains	This laboratory
Escherichia coli DH5α	EndA1 hsdR17($rk^- mk^+$) supE44 thi-1 recA gyrA relA1 ϕ80dlacZΔM15 Δ(lacZYA-argF)U169	Hannahan, 1983
Bacteriophage	**Characteristics**	**Source or reference**
KPP2, KPP3, KPP4, KPP5, KPP6, KPP7, KPP10, KPP11, KPP30, KPP42, KPP50 and KPP95	lytic phages of *Klebsiella pneumoniae*	This study
Plasmid	**Characteristics**	**Source or reference**
pOK12	*E. coli* general cloning vector, lacZ α fragment, P15A origin, Km^r, 2.1kb	Vieira and Messing, 1991

Table 2. Titer of 12 lytic phages.

Phage	Titer (PFU／ml) [a]
KPP2	2.8×10^9
KPP3	2.5×10^9
KPP4	2.7×10^9
KPP5	2.4×10^9
KPP6	3.5×10^9
KPP7	1.8×10^9
KPP10	1.55×10^9
KPP11	4×10^9
KPP30	1.6×10^9
KPP42	1.25×10^9
KPP50	1.5×10^9
KPP95	3.15×10^9

[a] The titers were measured using *K.pneumoniae* 10693 as the indicator host.

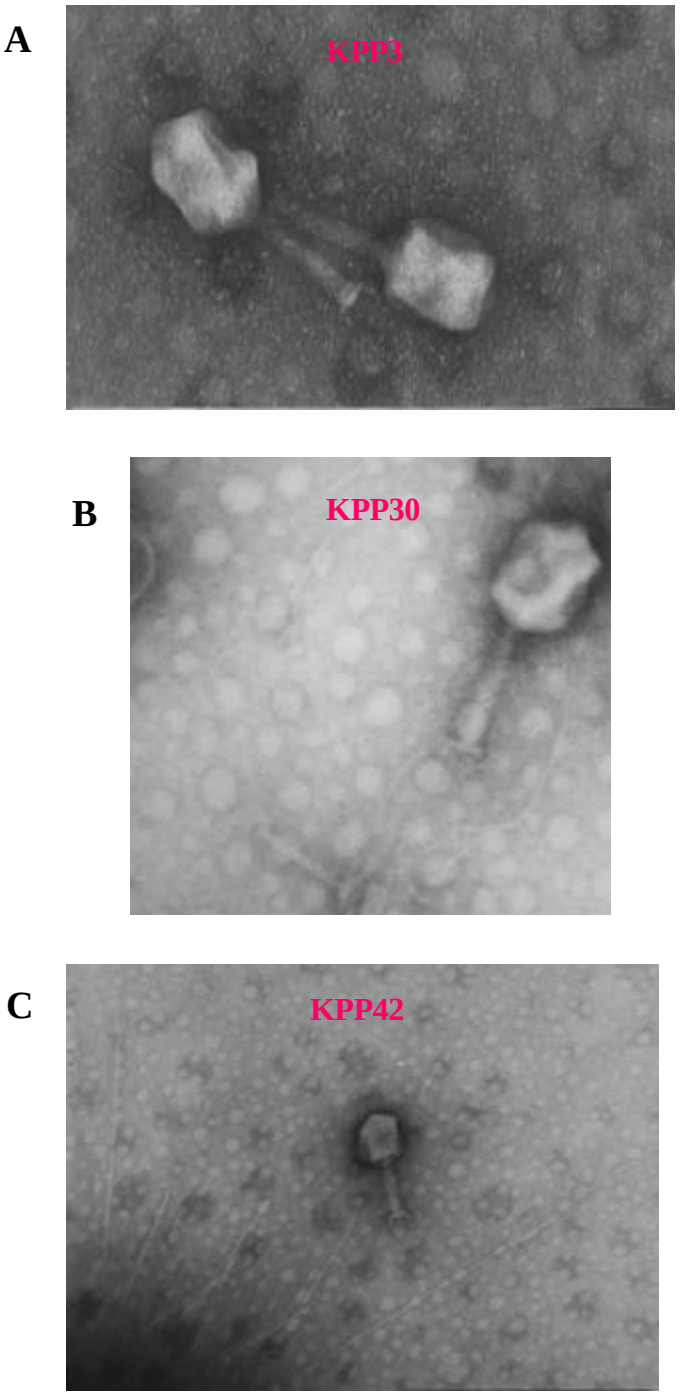
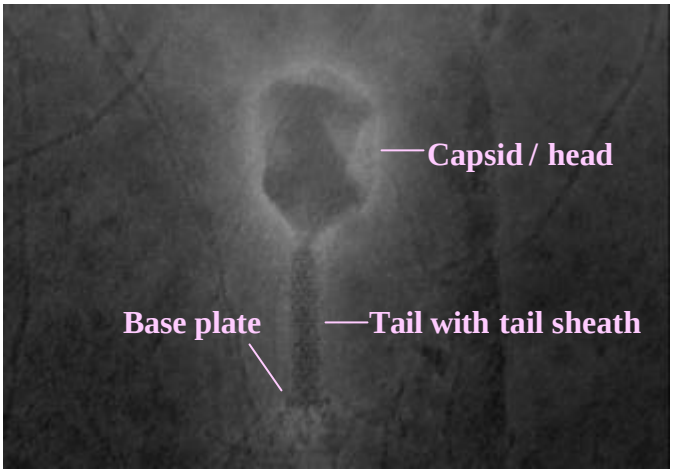
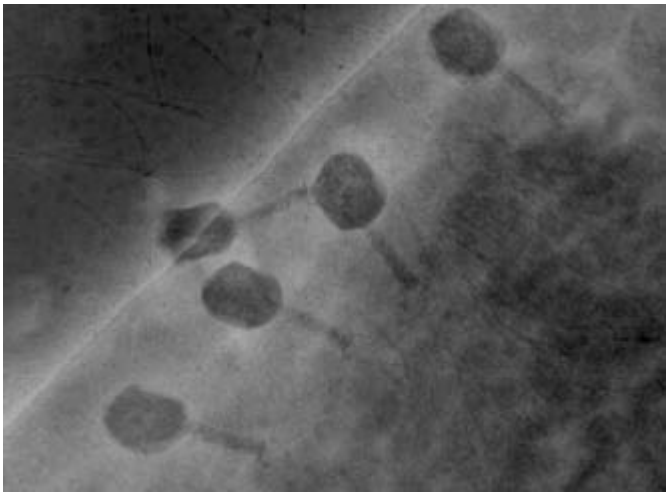


Fig. 1. Electron micrograph of four lytic phages of *K.pneumoniae* 10693 stained with 2 % Uranyl acetate. **A:** KPP3; B: KPP30; C: KPP42; D, E: KPP95. Magnification: A,B and C, 75000; D and E, 150,000.

D



E



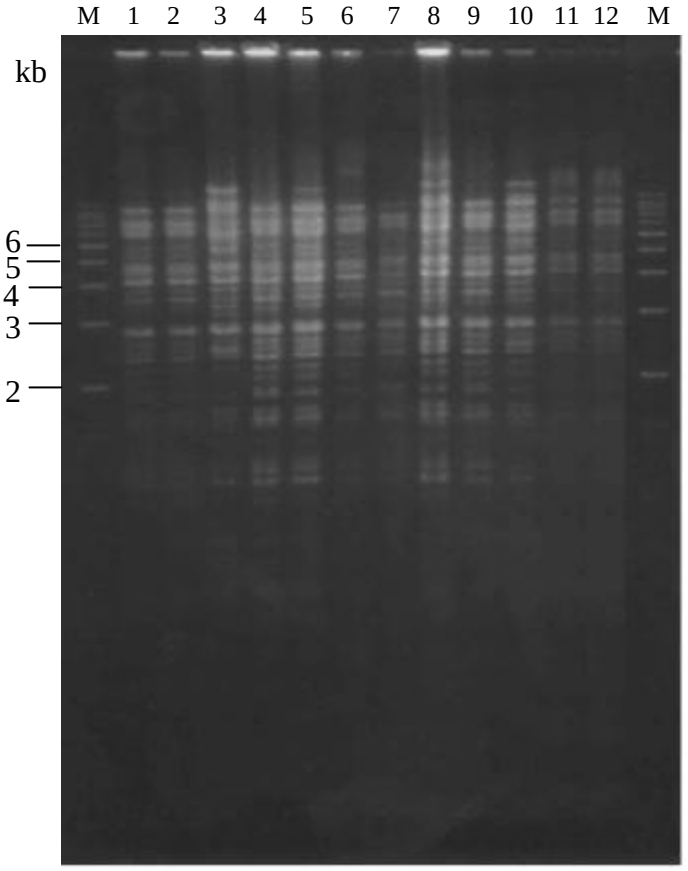


Fig. 2. Restriction fragment profiles of *Eco*RV-digested genomic DNA from KPP2, KPP3, KPP4, KPP5, KPP6, KPP7, KPP10, KPP11, KPP30, KPP42, KPP50 and KPP95.
Lanes: M, kb 25 markers ; 1, KPP2; 2, KPP3; 3, KPP4; 4, KPP5; 5, KPP6; 6, KPP7; 7, KPP10; 8, KPP11; 9, KPP30; 10, KPP42; 11, KPP50; 12, KPP95.

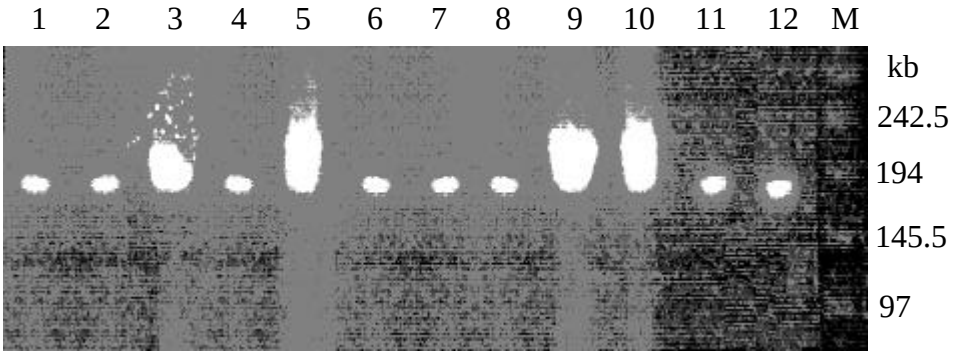


Fig. 3. Pulsed-field gel electrophoresis (PFGE) of KPP2, KPP3, KPP4, KPP5, KPP6, KPP7, KPP10, KPP11, KPP30, KPP42, KPP50 and KPP95 genomes. Electrophoresis was done in 1% agarose gel at 14℃ and 200 V, with initial time of 1 sec, final time of 40 sec and running time of 16 hr.

Lanes: M, λ ladder markers; 1, KPP2; 2, KPP3; 3, KPP4; 4, KPP5; 5, KPP6; 6, KPP7; 7, KPP10; 8, KPP11; 9, KPP30; 10, KPP42; 11, KPP50; 12, KPP95.

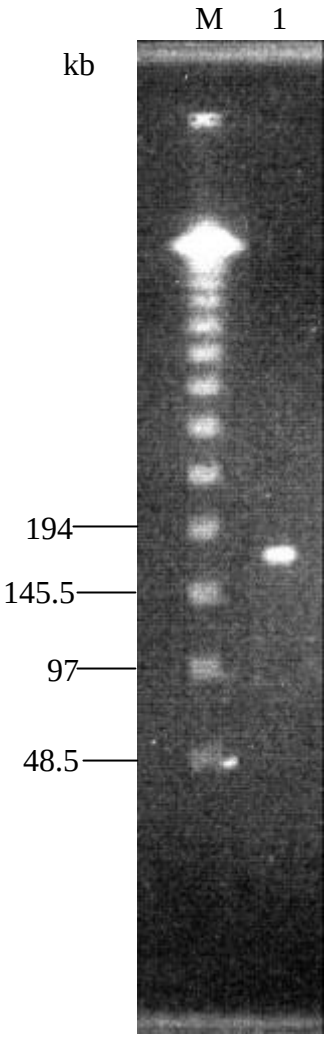


Fig. 4. Pulsed-field gel electrophoresis (PFGE) of KPP95 genome. Electrophoresis was done in 1% agarose gel at 14℃ and 200 V, with initial time of 1 sec, final time of 40 sec and running time of 20 hr. M, λ ladder markers.

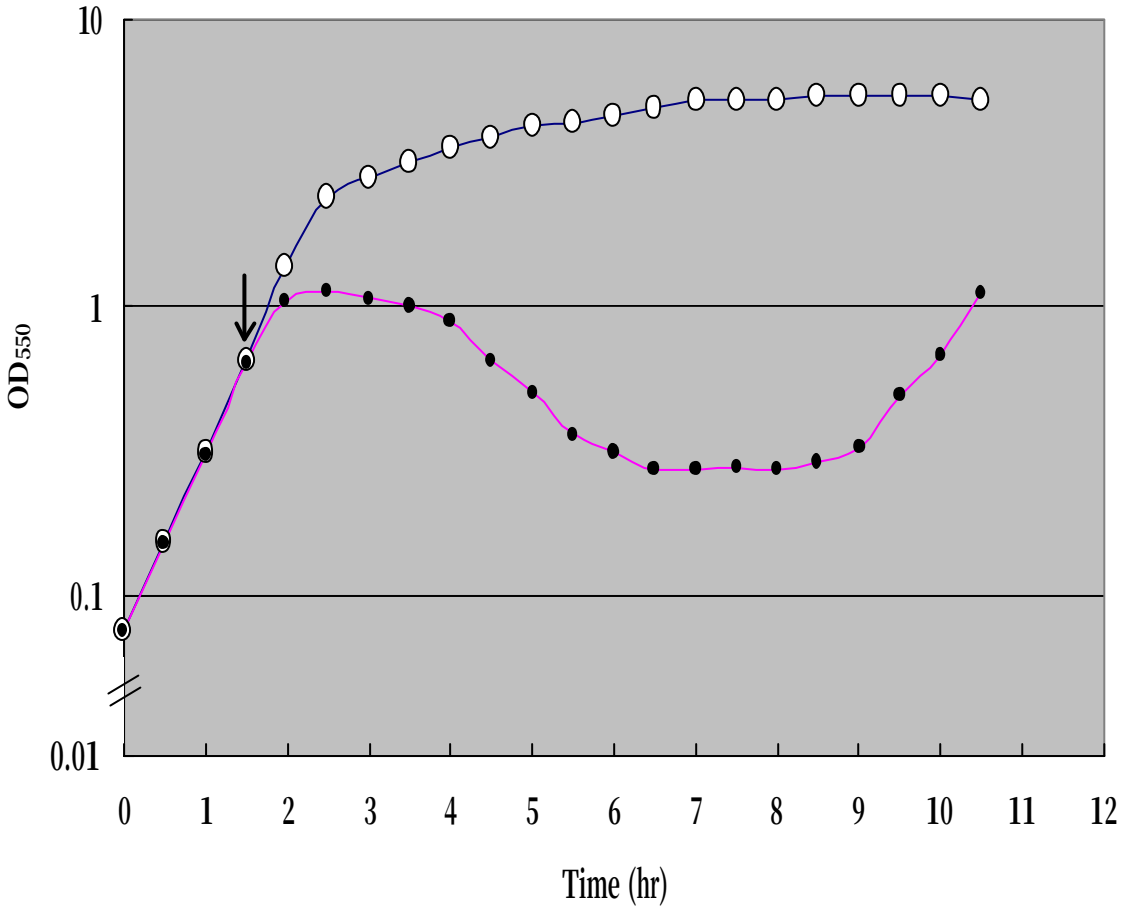


Fig.5. Lysis of Klebsiella pneumoniae 10693 by KPP95 phage. The Cultures with an initial OD_{550} of 0.05 were grown at 37℃, and the phage was added (MOI= 0.1) at 1.5 hr (indicated by arrow). Samples were taken for measurements of OD_{550}. ●, phage added; ○, without phage. Values are mean (n= 3).

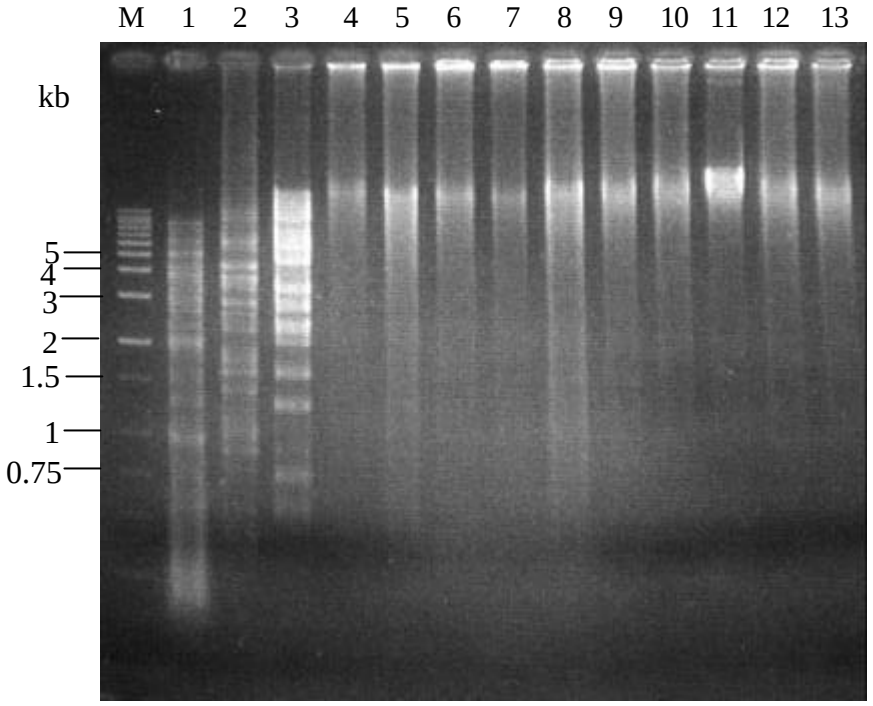


Fig. 6. Restriction endonuclease digestion of KPP95 genomic DNA.
Lanes: M, kb 25 markers；1, *Ssp*I；2, *Ase*I；3, *Dra*I；4, *Sma*I；5, *Nae*I；6, *Sac*II；7, *Bam*HI；8, *Sau*3A1；9, *Kpn*I；10, *Hae*III；11, *Ava*I；12, *Eco*RI；13, *Hin*dIII

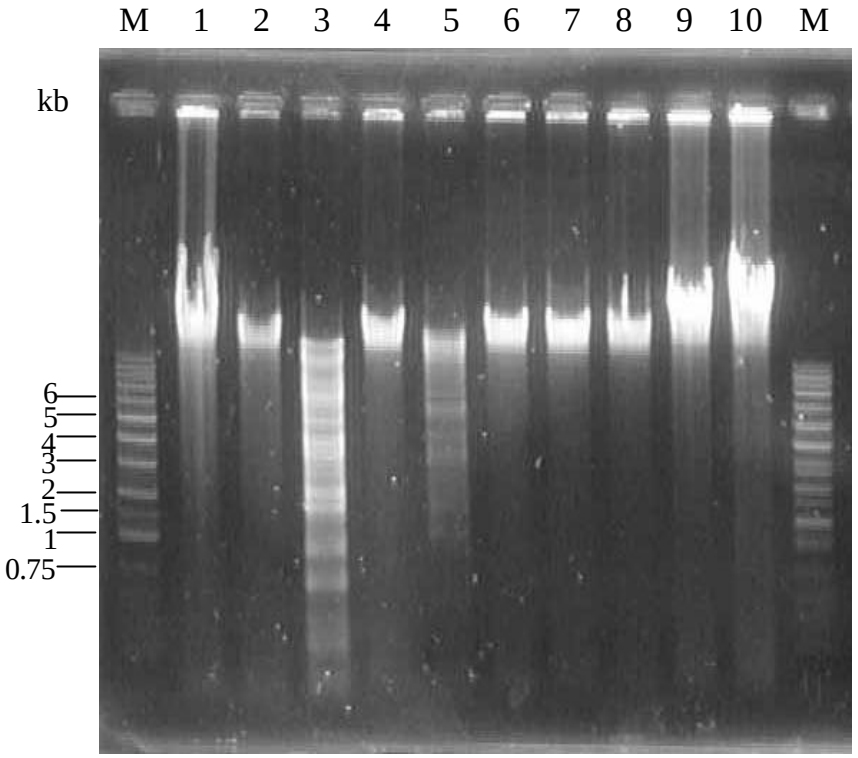


Fig. 7. Restriction endonuclease digestion of KPP95 genomic DNA.

Lanes: M, kb 25 markers；1, *Eco*RI；2, *Pst*I；3, *Eco*RV；4, *Hin*d**III**；5, *Nde*I；6, *Sca*I；7, *Acc*I；8, *Hpa*I；9, *Nsi*I；10, *Hin*cII

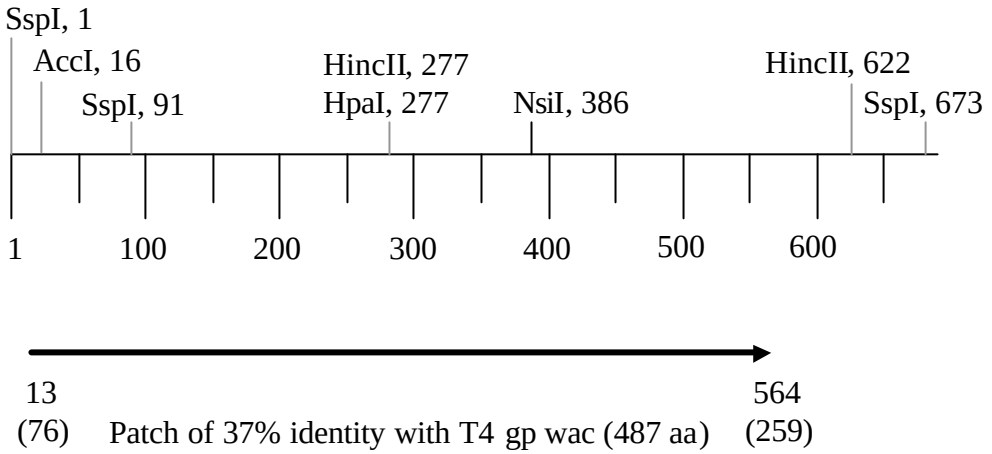


Fig. 8. Restriction map of 678 bps fragment cloned in pOS 1 and comparsion of putative polypeptide encoded by fragment 678 bps cloned in pOS 1 with protein of T4 phage. The corresponding position of pOS 1 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

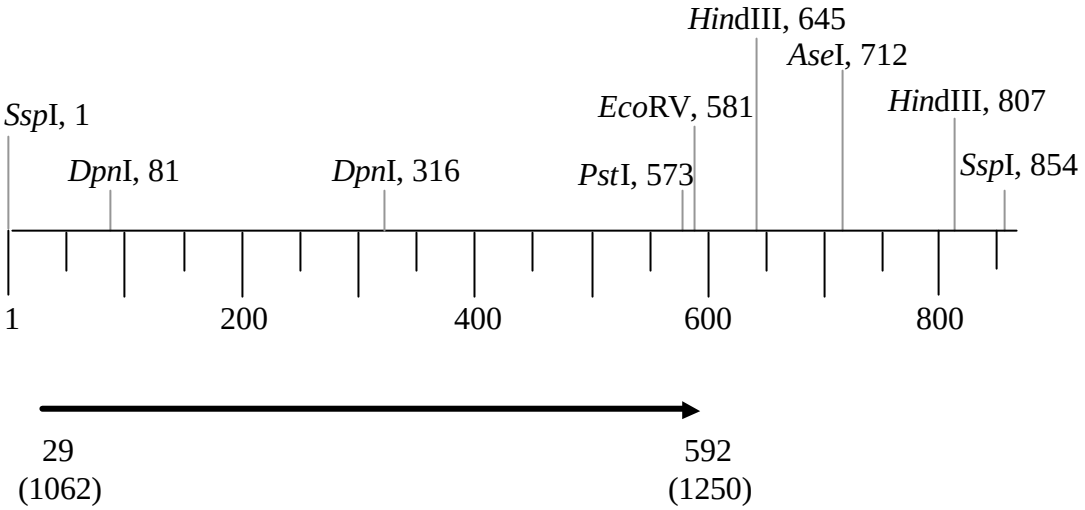


Fig. 9. Restriction map of 859 bps fragment cloned in pOS 2-34 and comparsion of putative polypeptide encoded by fragment 859 bps cloned in pOS 2-34 with protein of T4 phage. The corresponding position of pOS 2-34 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

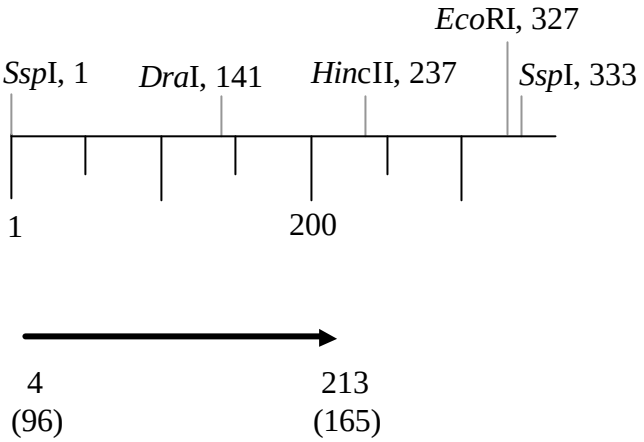


Fig. 10. Restriction map of 338 bps fragment cloned in pOS 2-37 and comparsion of putative polypeptide encoded by fragment 338 bps cloned in pOS 2-37 with protein of T4 phage. The corresponding position of pOS 2-37 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

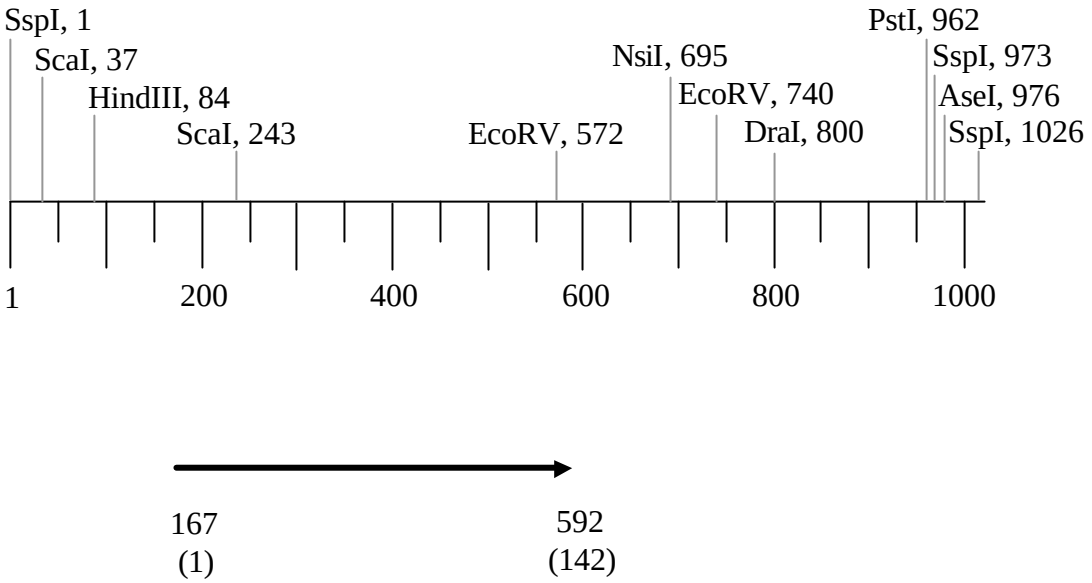


Fig. 11. Restriction map of 1031 bps fragment cloned in pOS 3 and comparsion of putative polypeptide encoded by fragment 1031 bps cloned in pOS 3 with protein of T4 phage. The corresponding position of pOS 3 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

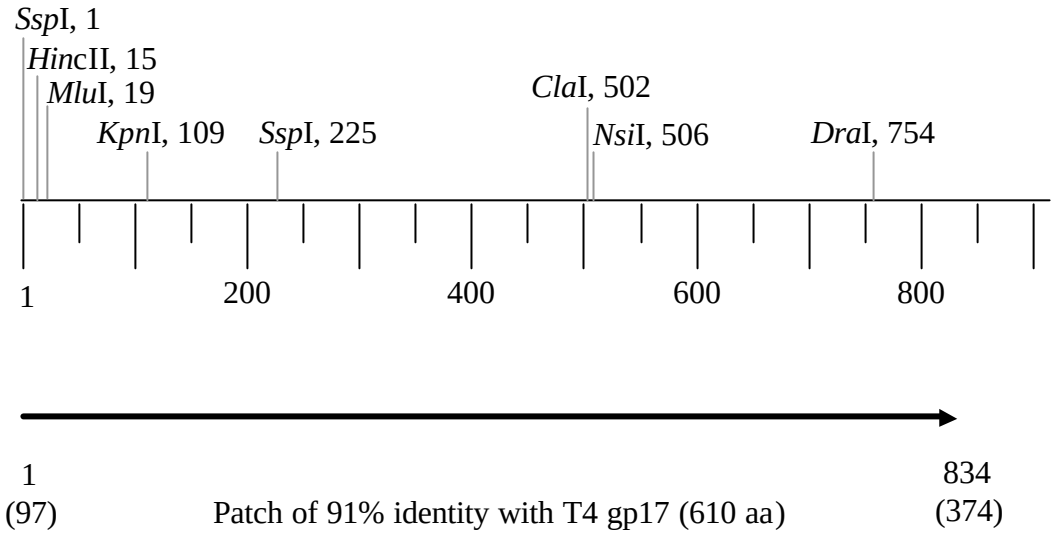


Fig. 12. Restriction map of 906 bps fragment cloned in pOS 11and comparsion of putative polypeptide encoded by fragment 906 bps cloned in pOS 11 with protein of T4 phage. The corresponding position of pOS 11was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

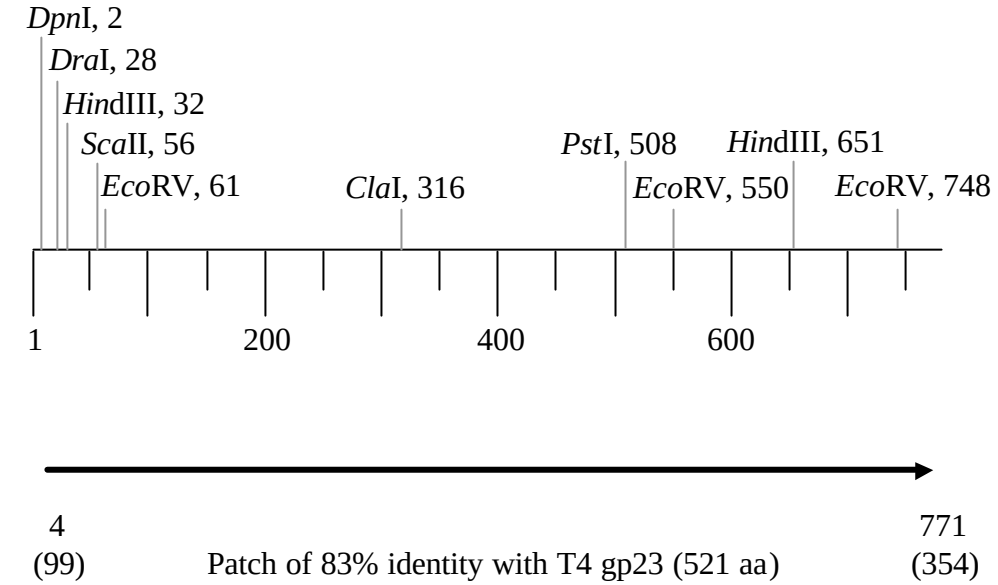


Fig.13. Restriction map of 789 bps PCR product-gene 23 fragment (PCR 23) and comparsion of putative polypeptide encoded by fragment 789 bps cloned in PCR 23 with protein of T4 phage. The corresponding position of PCR 23 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

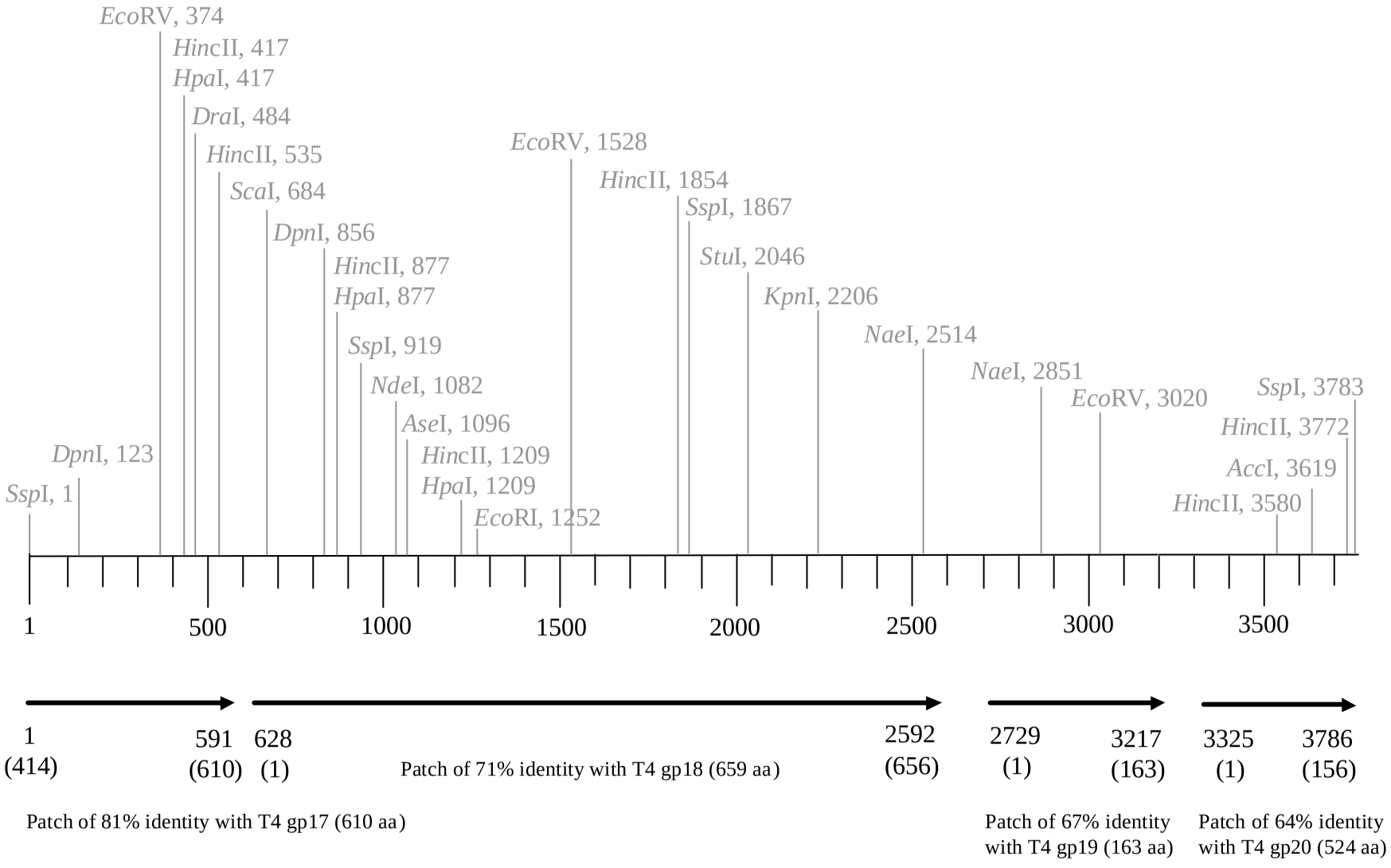


Fig. 14. Restriction map of 3788 bps fragment cloned in pOS 21 . and comparsion of putative polypeptide encoded by fragment 3788 bps cloned in pOS 21 with protein of T4 phage. The corresponding position of pOS 21 was represented by bps and that of the T4 phage protein was represented by (a.a.). The bar with arrow indicated the gene position.

Table 3. Comparsion of putative polypeptide encoded by fragment 678 bps cloned in pOS 1 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position Pos1 (bps) [a] Protein (aa) [b]
T4	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein) , 487 aa	37%	13 - 564 76 - 259
AR1	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein), 485 aa	36%	7 - 582 72 - 263
Ox2	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein), 487 aa	38%	13 - 564 76 - 259
RB43	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein), 762 aa	36%	4 - 669 60 - 300
RB49	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein), 589 aa	34%	16 - 669 78 - 294
Aeh1	Fibritin (gp wac) (Whisker antigen control protein) (Collar protein), 1035 aa	35%	217 - 558 137 - 250

The comparison are obtained by using NCBI blastx program, so the corresponding position of pOS 1 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

Table 4. Comparsion of putative polypeptide encoded by fragment 859 bps cloned in pOS 2-34 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 2-34 (bps) [a] Protein (aa) [b]
T4	Proximal tail fiber protein gp34, 1289 aa	27%	29 - 592 1062 - 1250
RB49	small distal tail fiber subunit Gp36 , 496 aa	33%	107 - 295 397 - 441

The comparison are obstained by using NCBI blastx program, so the corresponding position of pOS 2-34 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

Table 5. Comparsion of putative polypeptide encoded by fragment 338 bps cloned in pOS 2-37 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 2-37 (bps) [a] Protein (aa) [b]
T4	Long tail fiber protein gp37 (Receptor recognizing protein), 1026 aa	47%	4 - 213 96 - 165
K3	Long tail fiber protein gp37 (Receptor recognizing protein), 1243 aa	50%	43 - 213 110 - 166
PPO1	Long tail fiber protein gp37 (Receptor recognizing protein), 1109 aa	32%	7 - 258 104 - 183
Ac3	Long tail fiber protein gp37 (Receptor recognizing protein), 1103 aa	35%	7 - 258 104 - 183
AR1	Long tail fiber protein gp37 (Receptor recognizing protein), 1103 aa	29%	7 - 186 104 - 181

The comparison are obstained by using NCBI blastx program, so the corresponding position of pOS 2-37 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

Table 6. Comparsion of putative polypeptide encoded by fragment 1031 bps cloned in pOS 3 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 3 (bps) [a] Protein (aa) [b]
T4	Primase-helicase (Protein Gp41), 475 aa	68%	167 - 592 1 - 142
RB49	Primase-helicase (Protein Gp41), 470 aa	53%	167 - 592 1 - 142

The comparison are obstained by using NCBI blastx program, so the corresponding position of pOS 3 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

Table 7. Comparsion of putative polypeptide encoded by fragment 906 bps cloned in pOS 11 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 11 (bps) [a] Protein (aa) [b]
T4	DNA packaging protein Gp17 (Terminase) , 610 aa	91%	1 - 834 97 - 374
RB49	DNA packaging protein Gp17 (Terminase), 607 aa	76%	1 - 834 95 - 372
KVP40	DNA packaging protein Gp17 (Terminase) , 600 aa	60%	25 - 828 91 - 357
KVP20	DNA packaging protein Gp17 (Terminase), 600 aa	60%	22 - 828 90 - 357

The comparison are obstained by using NCBI blastx program, so the corresponding position of pOS 11 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

Table 8. Comparsion of putative polypeptide encoded by fragment 3788 bps cloned in pOS 21 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 21 (bps) [a] Protein (aa) [b]
T4	DNA packaging protein Gp17 (Terminase), 610 aa	81%	1 - 591 414 - 610
RB49	DNA packaging protein Gp17 (Terminase), 607 aa	70%	4 - 591 412 - 607
KVP40	DNA packaging protein Gp17 (Terminase) , 600 aa	51%	1 - 537 399 - 581
KVP20	DNA packaging protein Gp17 (Terminase) , 600 aa	51%	1 - 537 399 - 581

Table 9. Comparsion of putative polypeptide encoded by fragment 3788 bps cloned in pOS 21 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 21 (bps) [a] Protein (aa) [b]
T4	Portal vertex protein of head (Gp20), 524 aa	64%	3325 - 3786 1- 156
KVP40	Portal vertex protein of head (Gp20), 515 aa	39%	3337 - 3786 1 - 149
RB49	Portal vertex protein of head (Gp20), 99 aa	52%	3421 - 3708 2 - 98

Table 10. Comparsion of putative polypeptide encoded by fragment 3788 bps cloned in pOS 21 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 21 (bps) [a] Protein (aa) [b]
T4	Tail sheath protein (Gp18), 659 aa	71%	628 - 2592 1 - 656
42	Tail sheath protein (Gp18), 662 aa	69%	628 - 2595 1 - 659
RB42	Tail sheath protein (Gp18), 663 aa	64%	628 - 2598 1 - 661
RB49	Tail sheath protein (Gp18), 665 aa	64%	628 - 2595 1 - 661
KVP40	Tail sheath protein (Gp18), 671 aa	50%	628 - 2592 1 - 671
nt-1	Tail sheath protein (Gp18), 671 aa	49%	628 - 2592 1 - 671

Table 11. Comparsion of putative polypeptide encoded by fragment 3788 bps cloned in pOS 21 with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position pOS 21 (bps) [a] Protein (aa) [b]
T4	Tail tube protein (Gp19), 163 aa	67%	2729 - 3217 1 - 163
42	Tail tube protein (Gp19), 163 aa	69%	2729 - 3214 1 - 162
T6	Tail tube protein (Gp19), 163 aa	66%	2729 - 3217 1 - 163
RB49	Tail tube protein (Gp19), 164 aa	65%	2729 - 3217 3 - 164
KVP40	Tail tube protein (Gp19), 166 aa	60%	2729 - 3214 3 - 163
nt-1	Tail tube protein (Gp19), 166 aa	57%	2729 - 3214 3 - 163

The comparison are obstained by using NCBI blastx program, so the corresponding position of pOS 21 was represented by [a] bps and that of the phages protein was represented by [b] a.a.

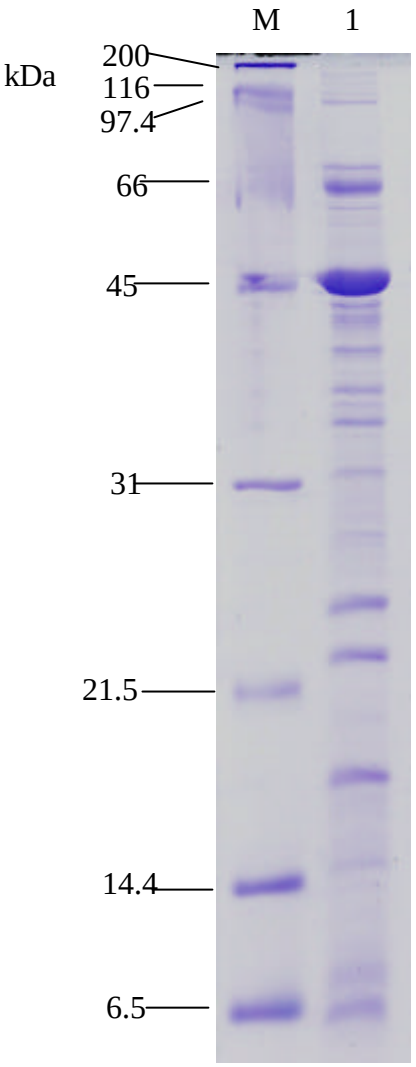


Fig. 15. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of KPP95 coat proteins.
M：BioRad broad range markers

Table 12. Comparsion of N-terminal sequence of 46 kDa putative major coat protein of KPP95 with that of different T4-type bacteriophages proteins.

Phage	Protein	Identity (%)	Corresponding N-terminal sequence (aa)
SV14	Major coat proteins (gp23), 503 aa	93	KPP95: 1 AEIGGDHGYDAQNIA 15 AEIGGDHGY+AQNIA SV14 : 67 AEIGGDHGYNAQNIA 81
RB69	Major coat proteins (gp23) , 512 aa	93	KPP95: 1 AEIGGDHGYDAQNIA 15 AEIGGDHGY AQNIA RB69 : 67 AEIGGDHGYXAQNIA 81
AR1	Major coat proteins (gp23) , 521 aa	86	KPP95: 1 AEIGGDHGYDAQNIA 15 AEIGGDHGY+A NIA AR1 : 66 AEIGGDHGYNATNIA 80
T4	Major coat proteins (gp23) , 521 aa	86	KPP95: 1 AEIGGDHGYDAQNIA 15 AEIGGDHGY+A NIA T4 : 66 AEIGGDHGYNATNIA 80
T6	Major coat proteins (gp23) , 502 aa	86	KPP95: 1 AEIGGDHGYDAQNIA 15 AEIGGDHGY+A NIA T6 : 66 AEIGGDHGYNATNIA 80

gene 23 fragment (PCR 23) with proteins of different T4-type bacteriophages.

Phage	Protein	Identity (%)	Corresponding position PCR 23 (bps) [a] Protein (aa) [b]
T4	major capsid protein (gp23), 521 aa	83%	4 - 771 99 - 354
T6	major capsid protein (gp23), 502 aa	82%	4 - 771 99 - 354
AR1	major capsid protein (gp23), 521 aa	82%	4 - 771 99 - 354
RB69	major capsid protein (gp23), 512 aa	81%	4 - 771 100 - 355
SV14	major capsid protein (gp23), 503 aa	80%	4 - 771 100 - 355
42	major capsid protein (gp23), 188 aa	80%	52 - 615 1 - 188
KC69	major capsid protein (gp23), 188 aa	78%	52 - 615 1 - 188
Tu1a	major capsid protein (gp23), 188 aa	78%	52 - 615 1 - 188
1	major capsid protein (gp23), 190 aa	75%	52 - 615 1 - 190
RB43	major capsid protein (gp23), 192 aa	66%	52 - 615 1 - 192
RB49	major capsid protein (gp23), 528 aa	64%	4 -771 98 - 362
44RR	major capsid protein (gp23), 197 aa	63%	52 - 615 1 - 197
65	major capsid protein (gp23), 193 aa	59%	52 - 615 1 - 193
KVP40	major capsid protein (gp23), 514 aa	57%	4 - 771 96 - 349
KVP20	major capsid protein (gp23), 514 aa	57%	4 - 771 96 - 349
Aeh1	major capsid protein (gp23), 193 aa	54%	52 - 615 1 - 193
nt-1	major capsid protein (gp23), 189 aa	53%	52 - 615 1 - 189

The comparison are obstained by using NCBI blastx program, so the opposite sequence of PCR 23 was represented by [a] bps and that of the phages protein was

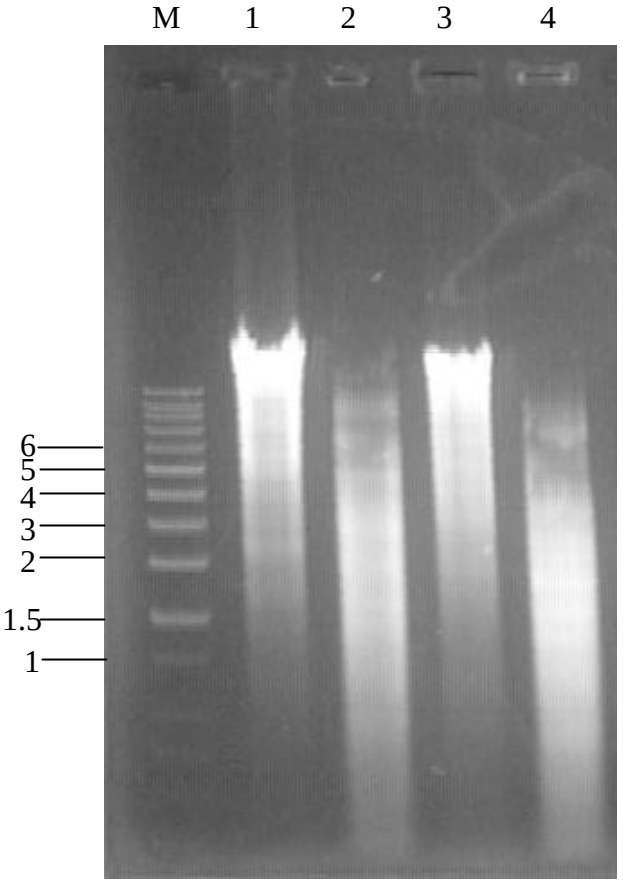


Fig. 16. Restriction enzyme digestion of *Klebsiella pneumoniae* 10693 chromosome
Lanes: M, kb 25 markers; 1, *Eco*RI; 2, *Pst*I; 3, *Hin*d**III**; 4, *Hin*cII

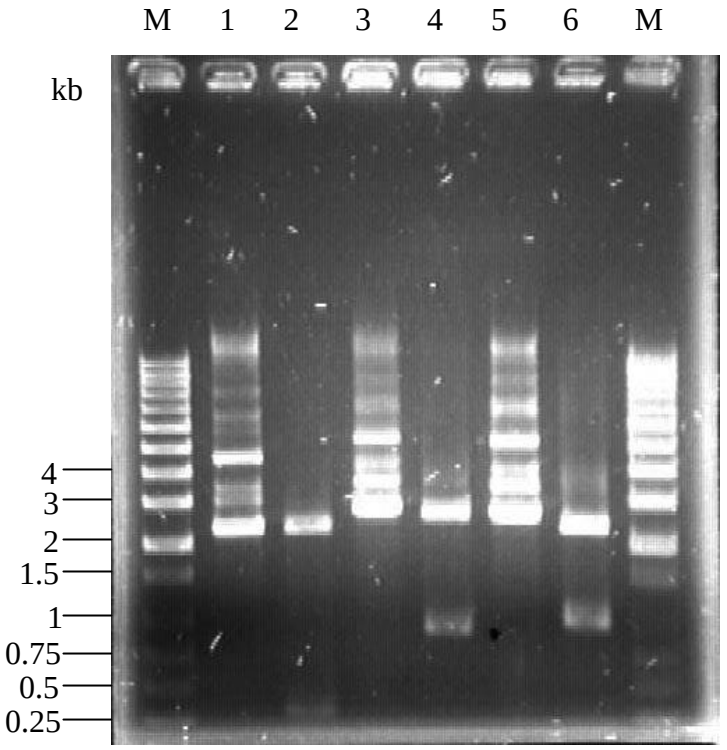


Fig. 17. Restriction enzyme digestion of plasmid DNA of three transformants pOS 1, pOS 2, pOS 3 (host, *E. coli* DH5 α) cloned from KPP95 genome.
Lanes: M, kb 25 markers; 1, pOS 1; 2, pOS 1 digested by *Hin*cII; 3, pOS 2; 4, pOS 2 digested by *Eco*RI; 5, pOS 3; 6, pOS 3 digested by *Pst*I

Recognition sequences contain either A or T only.

SspI	5’	AAT▼ATT	3’
	3’	TTA▲TAA	3’
AseI	5’	A▼TTAAT	3’
	3’	TAAT▲TA	5’
DraI	5’	TTT▼AAA	3’
	3’	AAA▲TTT	5’

Recognition sequences contain either G or C only.

SmaI	5’	CCC▼GGG	3’
	3’	GGG▲CCC	5’
NaeI	5’	GCC▼GGC	3’
	3’	CGG▲CCG	5’
SacII	5’	CCGC▼GG	3’
	3’	GG▲CGCC	5’

Not blocked by *dam* methylation

BamHI	5’	G▼GATCC	3’
	3’	CCTAG▲G	5’
Sau3A1	5’	▼GATC	3’
	3’	CTAG▲	5’

Not blocked by *dcm* methylation

KpnI	5’	GGTAC▼C	3’
	3 ’	C▲CATGG	5’
Hae**III**	5’	GG▼CC	3’
	3’	CC▲GG	5’

Others

AvaI	5’	C▼PyCGPuG	3’
	3’	GPuGCPy▲C	5’
EcoRI	5’	G▼AATTC	3’
	3’	CTTAA▲G	5’
Hind**III**	5’	A▼AGCTT	3’
	3’	TTCGA▲A	5’
*Eco*RI	5’	G▼AATTC	3’
	3’	CTTAA▲G	5’
*Pst*I	5’	CTGCA▼G	3’
	3’	G▲ACGTC	5’
*Eco*RV	5’	GAT▼ATC	3’
	3’	CTA▲TAG	5’
*Hin*d**III**	5’	A▼AGCTT	3’
	3’	TTCGA▲A	5’
*Nde*I	5’	CA▼TATG	3’
	3’	GTAT▲AC	5’
*Sca*I	5’	AGT▼ACT	3’
	3’	TCA▲TGA	5’
*Acc*I	5’	GT▼AT AC CG	3’
	3’	CA TA▲TG GC	5’
*Hpa*I	5’	GTT▼AAC	3’
	3’	CAA▲TTG	5’
*Nsi*I	5’	ATGCA▼T	3’
	3’	T▲ACGTA	5’
*Hinc*II	5’	GTP$_y$▼P$_u$AC	3’
	3’	CAP$_u$▲P$_y$TG	5’

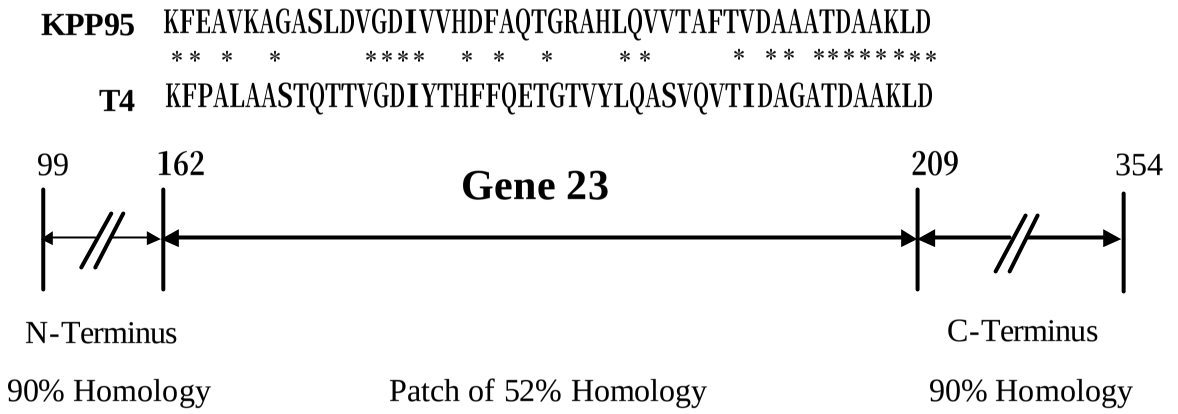


Fig. 19. Comparison between the sequences of gp23 in phages T4 and KPP95.
Asterisks between the alignments of the two sequences indicate identical residues in both genes.

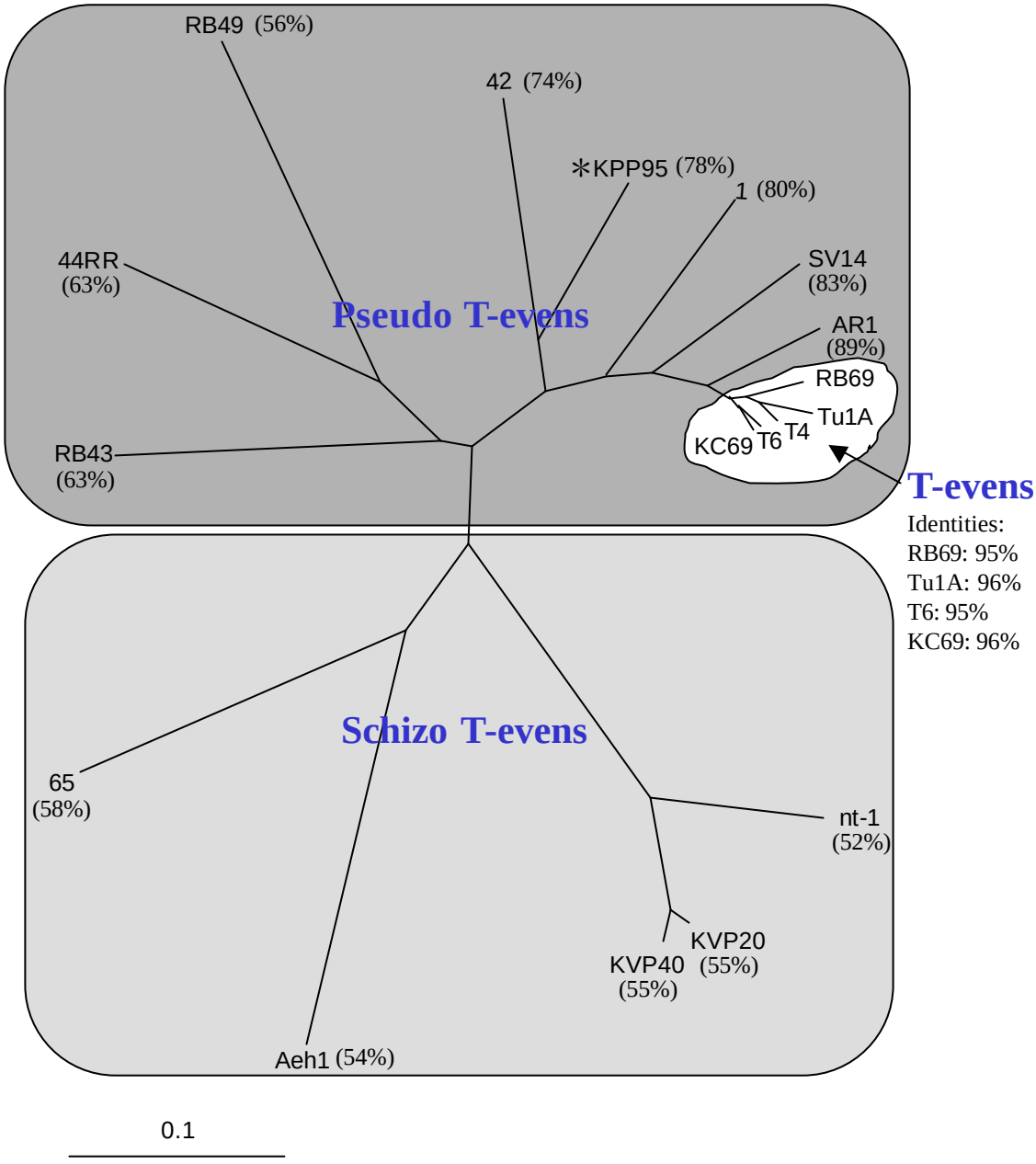


Fig. 22. Phylogenetic tree constructed by the TreeV32 program based on the sequence of gene 23 proteins from 18 T4-type phages. The percentages of aa identities between the T4 gene 23 sequence and that of the other T4-type phages are shown beside each branch of the tree. The varoius subgroups are indicated by the shaded areas (white, T-evens; dark gray, pseudo T-evens; and light gray, schizo T-evens).