Methods

Biofilm formation assay

CRKP cells were inoculated into 5 mL of sterile LB broth and grown for 16 h at 37°C. A total of 200 μL of each diluted culture was subsequently added to the wells of untreated 96-well microtiter plates. A total of 200 μL of fresh LB broth was used as a negative control. The plates were incubated at 37°C for 48 h without agitation. Nonadherent cells were removed by pipetting the culture and washing the wells three times with 200 μL of sterile PBS. Then, a total of 200 μL of 99% methanol was added to each well, and the plates were incubated for 15 min at room temperature. The methanol was removed, and the plates were dried at room temperature. A total of 200 μL of 1% w/v crystal violet (CV) solution was subsequently added to each well, after which the plates were incubated for 30 min at room temperature. The wells were then washed gently with sterile PBS and left to dry. Next, a total of 160 μL of 33% v/v glacial acetic acid was added to each well to solubilize the bound CV from the stained CRKP biofilm. The OD_{570} was measured via a spectrophotometer from Thermo Fisher Scientific (China) Co., Ltd.

Determination of the effect of phage on CRKP biofilms

The CRKP strain 21AA2216 was used to test the prophylactic effect of the phage on biofilm formation. Briefly, a total of 100 μL of CRKP cells were added to each well of a 96-well microtiter plate. The wells were subsequently divided into five groups. Phage solutions with different MOI ratios of 0.1, 0.01, 0.001, and 0.0001 were added to the

different groups in a volume of 100 μL; furthermore, a total of 100 μL of CRKP cells and 100 μL of sterile LB broth were used as a control. The plate was covered, and the CRKP cells were allowed to adhere and grow at 37°C for 6, 12, 24, and 48 h without agitation. After incubation, the residual biofilm was stained, and the OD_{570} was measured.

Biofilm formation was carried out following the previously described to assess the efficacy of phage-breaking CRKP mature biofilms. In brief, biofilms were cultivated in fresh LB in a 96-well microtitration plate at 37°C for a duration of 48 h. The biofilm was washed with sterile PBS, and phage solutions were added at MOI ratios of 0.1, 0.01, 0.001, and 0.0001 for 4 h at 37°C. LB broth was used as the control. After treatment, the residual biofilm was stained, and the OD_{570} was determined.

Morphological analyses were conducted via cold field emission SEM at the Institute of Microbiology, Chinese Academy of Sciences. The CRKP biofilms in the experimental and control groups were treated with either phage or LB broth, respectively. The biofilms were rinsed three times using sterile PBS. They were then immobilized with 2.5% glutaraldehyde overnight at 4°C, and the cells were dehydrated with different concentrations of ethanol. Subsequently, tert-butanol was added to freeze overnight at −80°C. Afterwards, the samples on cover glasses were freeze-dried and sputter coated with gold. Finally, the surface morphology of the CRKP cells was examined via SEM.

To determine the number of viable cells, the experimental group was prepared by treating the CRKP biofilm with phage medium. The phage-untreated biofilm growth control of CRKP in LB broth was also prepared. The adhered cells were scraped from the bottom and sides of the wells with a sterile pipette tip and then suspended in 9 mL of sterile PBS. The suspension was then vigorously vortexed for 1 min. Serial dilutions of these cell suspensions were made and spread onto LB agar plates. The colonies formed were counted and expressed as log10 CFU/mL, considering the dilution factor.

Expression and purification of the phage depolymerase

The gene encoding the putative phage depolymerase ORF17 was amplified from the purified phage vB_KpnM_JYSS3 via PCR using the primers ORF17 PF (5'-CGGGATCCCGATGACCATTATCAAACGCG-3') and ORF17 PR (5'-CCGCTCGAGCGGCTATGTAAAATTAATAATGAAATTATCGACGTC-3'). The PCR fragment was digested with BamHI and XholI (New England Biolabs, USA) and inserted into the pET-28a expression vector (Sangon Biotech, China). The constructed plasmid was used to transform into *Escherichia coli* BL21 (DE3) cells (Sangon Biotech, China). The ORF17 protein was expressed under 0.5 mM isopropyl *β*-D1-thiogalactopyranoside (IPTG) induction overnight at 16°C, purified from the soluble fraction via a Ni-nitrilotriacetic acid (NTA) column (Sangon Biotech, China) according to the manufacturer's instructions, and then analysed via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Tests of the activity of depolymerase against CPS and biofilms

A total of 100 μL of sterile PBS, 10 μg/well depolymerase, or 1 μg/well depolymerase was added to the different groups, and 200 μL of sterile LB broth was used as the control. Each group had three replicate samples. The plate was covered, and the CRKP cells were allowed to adhere and grow at 37°C for 48 h without agitation. After incubation, the residual biofilm was stained, and the OD_{570} was measured. The effects of depolymerase on 48 h biofilms were also assessed. The biofilms were treated with 10 μg/well depolymerase, 1 μg/well depolymerase, or sterile PBS for 4 h, and 200 μL of sterile LB broth was used as a control. Each group had three replicate samples. After incubation, the residual biofilm was stained, and the OD_{570} was measured.

Table S1 The host range of phage vB_KpnM_JYSS3 and depolymerase Dep17

Bacterial strain	Source	Serotype	Plaque assay	Hole assay	Note
21AA0009	Urine	K64	−	−	CRKP
21AA0010	Sputum	K47	−	−	CRKP
21AA0026	Sputum	K2	−	+	CRKP
21AA0029	Sputum	K57	−	−	CRKP
21AA0131	Sputum	K19	−	−	CRKP
21AA0160	Sputum	K19	−	−	CRKP
21AA0218	Sputum	K19	−	−	CRKP
21AA0275	Urine	K47	−	−	CRKP
21AA0388	Sputum	K64	−	−	CRKP
21AA0398	Sputum	K19	−	−	CRKP
21AA0520	Sputum	K19	−	−	CRKP
21AA0600	Sputum	K25	−	−	CRKP
21AA0662	Sputum	K64	−	−	CRKP
21AA0714	Sputum	K106	−	−	CRKP
21AA0786	Sputum	K64	−	−	CRKP
21AA0828	Sputum	K25	−	−	CRKP
21AA0842	Urine	K64	−	−	CRKP
21AA0863	Sputum	K64	−	−	CRKP
21AA1035	BALF	K64	−	−	CRKP
21AA1042	Sputum	K64	−	−	CRKP
21AA1074	Sputum	K64	−	−	CRKP
21AA1100	Sputum	K64	−	−	CRKP
21AA1110	Sputum	K64	−	−	CRKP
21AA1115	Urine	K55	−	−	CRKP
21AA1117	Sputum	K64	−	−	CRKP
21AA1334	Urine	K47	−	−	CRKP
21AA1746	Effusion	K64	−	−	CRKP

21AA1795	Urine	K47	−	−	CRKP
21AA1819	Sputum	K64	−	−	CRKP
21AA1830	Sputum	K47	−	−	CRKP
21AA1842	Sputum	K64	−	−	CRKP
21AA1922	Sputum	K64	−	−	CRKP
21AA1942	Secretion	K64	−	−	CRKP
21AA1978	Effusion	K64	−	−	CRKP
21AA1997	Secretion	K64	−	−	CRKP
21AA2204	Effusion	K64	−	−	CRKP
21AA2205	Blood	K64	−	−	CRKP
21AA2206	Sputum	K64	−	−	CRKP
21AA2207	Sputum	K64	−	−	CRKP
21AA2210	Urine	K64	−	−	CRKP
21AA2211	Sputum	K64	−	−	CRKP
21AA2212	Sputum	K27	−	−	CRKP
21AA2213	Sputum	K64	−	−	CRKP
21AA2214	Urine	K64	−	−	CRKP
21AA2216	Sputum	K2	+	+	CRKP
21AA2217	Sputum	K64	−	−	CRKP
21AA2218	Sputum	K64	−	−	CRKP
21AA2219	Sputum	K64	−	−	CRKP
21AA2220	Sputum	K167	−	−	CRKP
21AA2221	Sputum	K64	−	−	CRKP
21AA2222	Sputum	K64	−	−	CRKP
21AA2224	Effusion	K64	−	−	CRKP
21AA2225	Effusion	K64	−	−	CRKP
21AA2226	Sputum	K23	−	−	CRKP
21AA2227	Sputum	K64	−	−	CRKP
21AA2228	Urine	K64	−	−	CRKP

21AA2244	Sputum	K64	—	—	CRKP
21AA2271	Secretion	K64	—	—	CRKP
21AA2414	Sputum	K64	—	—	CRKP
21AA2494	Sputum	K19	—	—	CRKP
21AA2588	Sputum	K64	—	—	CRKP
21AA2657	BALF	K64	—	—	CRKP
21AA2755	Sputum	K64	—	—	CRKP
21AA2768	Sputum	K19	—	—	CRKP
21AA2861	Urine	K64	—	—	CRKP
21AA2926	Sputum	K149	—	—	CRKP
21AA2946	BALF	K19	—	—	CRKP
21AA2948	Sputum	K64	—	—	CRKP
21AA2972	Blood	K19	—	—	CRKP
21AA3004	Sputum	K57	—	—	CRKP
21AA3032	Urine	K125	—	—	CRKP
21AA3151	Urine	K64	—	—	CRKP
21AA3165	Secretion	K102	—	—	CRKP
21AA3170	Sputum	K64	—	—	CRKP
22AA0227	BALF	K47	—	—	CRKP
22AA0324	Sputum	K47	—	—	CRKP
22AA0608	Sputum	K28	—	—	CRKP
22AA0727	Urine	K64	—	—	CRKP
22AA1153	Urine	K47	—	—	CRKP
22AA1241	Effusion	K64	—	—	CRKP
22AA1259	BALF	K19	—	—	CRKP
22AA1326	Sputum	K19	—	—	CRKP
22AA1424	Urine	K47	—	—	CRKP
22AA1455	Urine	K64	—	—	CRKP
22AA1717	Sputum	K102	—	—	CRKP

22AA1722	Urine	K64	—	—	CRKP
22AA1847	Sputum	K19	—	—	CRKP
22AA1918	Sputum	K19	—	—	CRKP
22AA2082	Blood	K19	—	—	CRKP
22AA2263	Urine	K24	—	—	CRKP
22AA2346	BALF	K19	—	—	CRKP
22AA2528	Urine	K19	—	—	CRKP
22AA2629	Sputum	K19	—	—	CRKP
22AA2700	Blood	K47	—	—	CRKP
22AA2705	Secretion	K47	—	—	CRKP
22AA3980	Sputum	K19	—	—	CRKP

Note: +, lyse; —, not lyse.

Table S2 The MOI of phage vB_KpnM_JYSS3

Tube	Phage titer (PFU/mL)	Host bacterial concentration (CFU/mL)	Multiplicity of infection	Titer at 9h (PFU/mL)
1	1×10^{9}	1×10^{7}	100	3.3×10^{9}
2	1×10^{8}	1×10^{7}	10	7.2×10^{9}
3	1×10^{7}	1×10^{7}	1	1.1×10^{10}
4	1×10^{6}	1×10^{7}	0.1	1.2×10^{10}
5	1×10^{5}	1×10^{7}	0.01	5.4×10^{9}
6	1×10^{4}	1×10^{7}	0.001	5.7×10^{9}
7	1×10^{3}	1×10^{7}	0.0001	3.7×10^{10}
8	1×10^{2}	1×10^{7}	0.00001	1.6×10^{10}
9	1×10^{1}	1×10^{7}	0.000001	1.6×10^{10}

Table S3 The characteristics of phages closely related to vB_KpnM_JYSS3

Phage	RCIP0093	vB_KpnM_15-38_KLPPOU148	vB_KpnM_JustaPhage	vB_KpnM_FZ14	KpTDp1
Nucleic acid type	DNA linear	DNA linear	DNA linear	DNA circular	DNA linear
Host strain	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*
Accession number	OR532887	NC_071128	NC_071134	NC_071133	PP847084
Total length (bp)	46213	49169	48129	49370	49311
Identity	96.80%	92.47%	91.73%	91.52%	90.78%
Query Cover	96%	76%	73%	73%	72%
Intergenomic similarity	95.5%	72.9%	69.6%	67.9%	67.7%
Taxonomy	—	*Jedunavirus*	*Jedunavirus*	*Jedunavirus*	*Jedunavirus*

Phage	MEW1	RCIP0063	vB_KpnM_IME346	RCIP0045	vB_KpnM_KpV52
Nucleic acid type	DNA linear	DNA linear	DNA linear	DNA linear	DNA linear
Host strain	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*
Accession number	NC_071135	OR532857	NC_071137	OR532839	NC_041900
Total length (bp)	47129	47703	49482	48709	47405
Identity	91.19%	90.85%	92.64%	91.22%	90.71%
Query Cover	70%	67%	67%	66%	64%
Intergenomic similarity	66.3%	65.2%	64%	62.1%	62.5%
Taxonomy	*Jedunavirus*	—	*Jedunavirus*	—	*Jedunavirus*

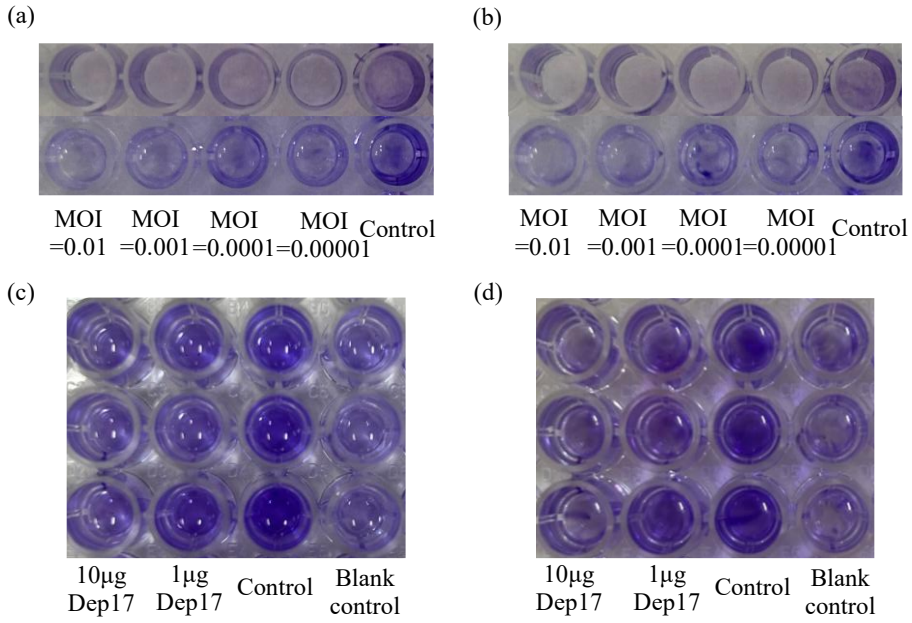
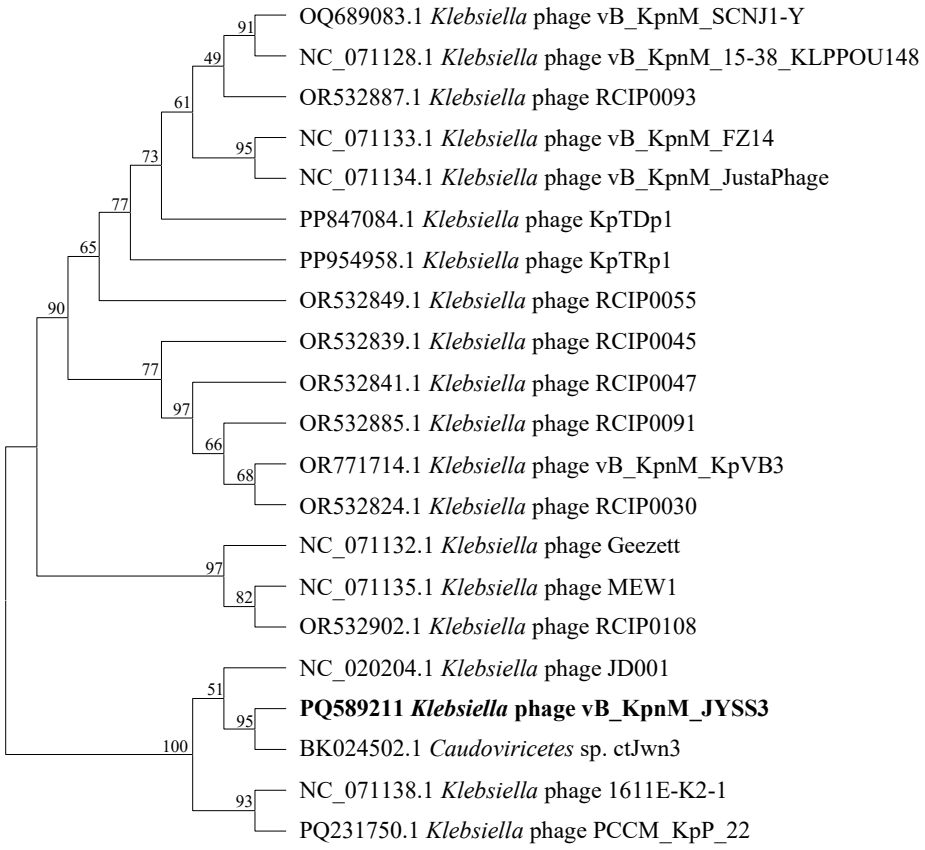


Fig. S1 The images of the residual biofilm by crystal violet staining

Note: a, CV staining proved the phage can inhibit biofilm formed by CRKP; b, the phage can destroy the mature biofilm; c, Dep17 can inhibit biofilm; d, Dep17 can destroy the mature biofilm.

(a)


OQ689083.1 *Klebsiella* phage vB_KpnM_SCNJ1-Y
NC_071128.1 *Klebsiella* phage vB_KpnM_15-38_KLPPOU148
OR532887.1 *Klebsiella* phage RCIP0093
NC_071133.1 *Klebsiella* phage vB_KpnM_FZ14
NC_071134.1 *Klebsiella* phage vB_KpnM_JustaPhage
PP847084.1 *Klebsiella* phage KpTDp1
PP954958.1 *Klebsiella* phage KpTRp1
OR532849.1 *Klebsiella* phage RCIP0055
OR532839.1 *Klebsiella* phage RCIP0045
OR532841.1 *Klebsiella* phage RCIP0047
OR532885.1 *Klebsiella* phage RCIP0091
OR771714.1 *Klebsiella* phage vB_KpnM_KpVB3
OR532824.1 *Klebsiella* phage RCIP0030
NC_071132.1 *Klebsiella* phage Geezett
NC_071135.1 *Klebsiella* phage MEW1
OR532902.1 *Klebsiella* phage RCIP0108
NC_020204.1 *Klebsiella* phage JD001
PQ589211 *Klebsiella* phage vB_KpnM_JYSS3
BK024502.1 *Caudoviricetes* sp. ctJwn3
NC_071138.1 *Klebsiella* phage 1611E-K2-1
PQ231750.1 *Klebsiella* phage PCCM_KpP_22
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68
97
82
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95
100
93

(b)

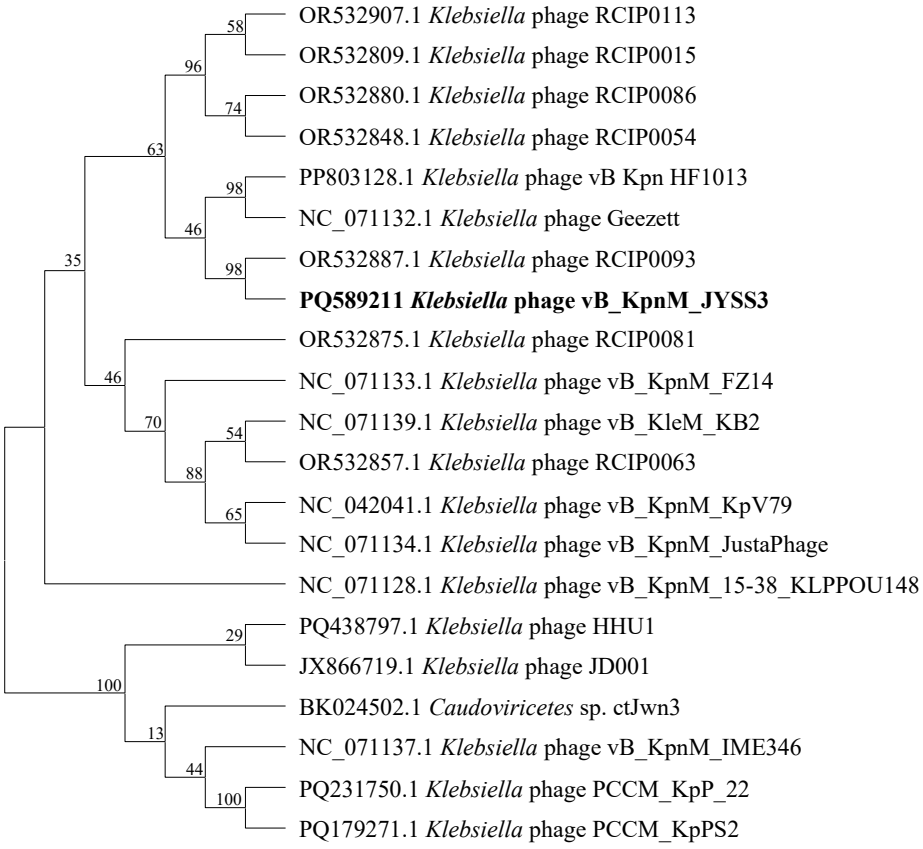

Fig. S2 Phylogenetic analysis results

Note: a, a phylogenetic tree based on the DNA polymerase sequences of vB_KpnM_JYSS3 and related *Klebsiella* phages using the maximum-likelihood method with 1000 bootstrap replicates; b, a phylogenetic tree based on the gene sequences of terminase large subunit of vB_KpnM_JYSS3 and related phages using the maximum-likelihood method with 1000 bootstrap replicates.

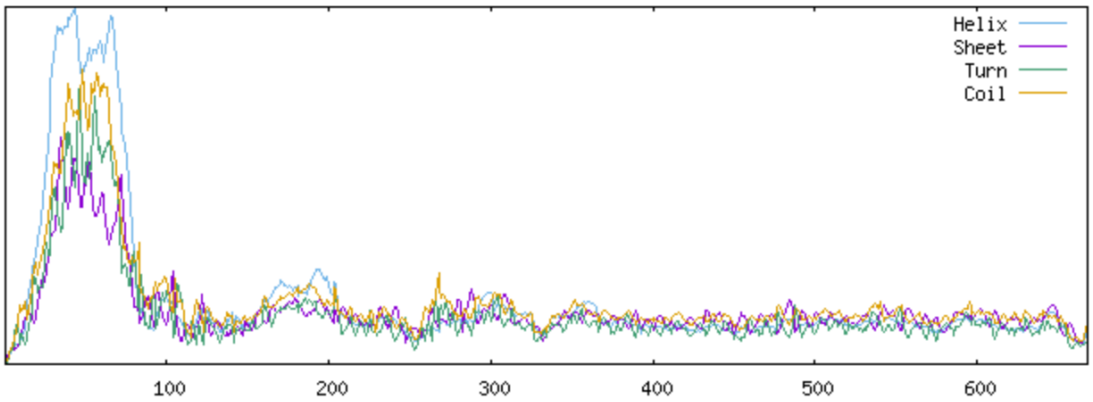

Fig. S3 The presence of secondary structures at different positions in the amino acid sequence

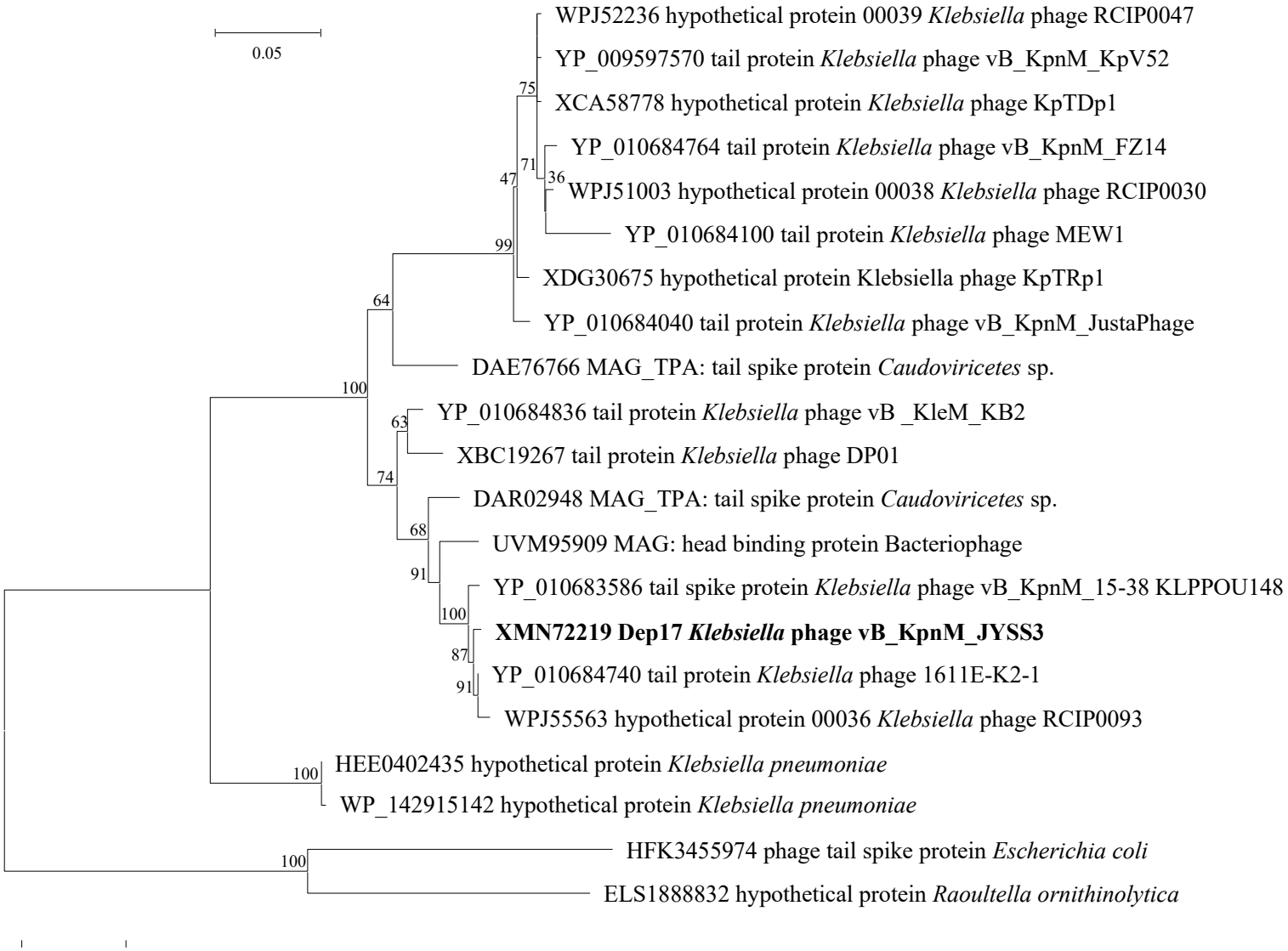

Fig. S4 A phylogenetic tree based on the amino acid sequences of Dep17 and its aligned sequences using the maximum-likelihood method with 1000 bootstrap replicates

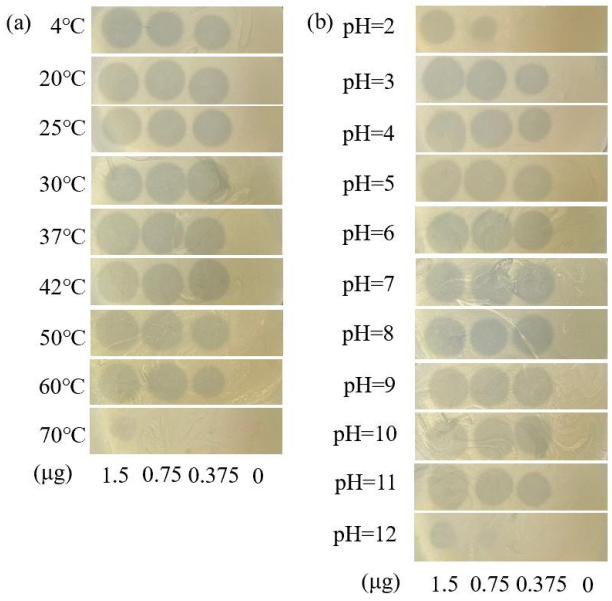

Fig. S5 Stability and activity of Dep17

Note: a, effect of temperature on Dep17 stability and activity. Different concentrations of Dep17 were preincubated at different temperatures for 1 h, and the residual activity was measured; b, stability, and activity of Dep17 under different pH buffers. Different concentrations of Dep17 were preincubated in different pH buffers for 1 h, and the residual activity was measured.

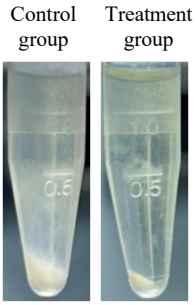

Fig. S6 CPS degradation activity of Dep17

Note: centrifugal precipitation of strain in the absence and presence of Dep17.