

TABLE S1. Host spectrum of phage KHP30 among *H. pylori* strains.

<i>H. pylori</i> strain	Sensitivity to phage KHP30 ^a	Description of <i>H. pylori</i> strain	Reference	Typing by RFLP ^b	
				UreAB PCR/HaeIII	FlaA PCR/HhaI
KMT7	-	Isolated at Kochi, Japan	This study	A	1
KMT9	+	Isolated at Kochi, Japan	This study	B	2
KMT15	-	Isolated at Kochi, Japan	This study	C	3
KMT16	-	Isolated at Kochi, Japan	This study	C	3
KMT17	-	Isolated at Kochi, Japan	This study	D	2
KMT18	+	Isolated at Kochi, Japan	This study	E	3
KMT19	-	Isolated at Kochi, Japan	This study	D	2
KMT28	-	Isolated at Kochi, Japan	This study	F	1
KMT84	+	Isolated at Kochi, Japan	This study	C	2
KMT85	+	Isolated at Kochi, Japan	This study	I	3
KMT86	+	Isolated at Kochi, Japan	This study	C	2
KMT87	+	Isolated at Kochi, Japan	This study	C	1
KMT88	-	Isolated at Kochi, Japan	This study	J	6
KMT89	+	Isolated at Kochi, Japan	This study	D	3
KMT90	-	Isolated at Kochi, Japan	This study	K	7
KMT114	+	Isolated at Kochi, Japan	This study	L	3
KMT117	-	Isolated at Kochi, Japan	This study	G	1
KMT124	-	Isolated at Kochi, Japan	This study	F	1
KMT126	+	Isolated at Kochi, Japan	This study	L	2
KMT130	+	Isolated at Kochi, Japan	This study	K	3
NY1	-	Isolated at Yamaguchi, Japan	This study	L	5
NY2	+	Isolated in Yamaguchi, Japan	This study	M	1
NY3	+	Isolated in Yamaguchi, Japan	This study	C	6
NY4	+	Isolated in Yamaguchi, Japan	This study	C	6
NY5	+	Isolated in Yamaguchi, Japan	This study	N	2
NY6	+	Isolated in Yamaguchi, Japan	This study	N	2
NY9	+	Isolated in Yamaguchi, Japan	This study	H	2
NY10	-	Isolated in Yamaguchi, Japan	This study	D	7
NY11	+	Isolated in Yamaguchi, Japan	This study	O	8
NY12	+	Isolated in Yamaguchi, Japan	This study	O	8
NY23	+	Isolated in Yamaguchi, Japan	This study	H	5
NY24	-	Isolated in Yamaguchi, Japan	This study	J	2
NY31	-	Isolated in Yamaguchi, Japan	This study	D	5
NY37	+	Isolated in Yamaguchi, Japan	This study	G	4
NY38	+	Isolated in Yamaguchi, Japan	This study	H	5
NY43	-	Isolated in Yamaguchi, Japan	This study	P	7
NY44	+	Isolated in Yamaguchi, Japan	This study	P	7
NY50	+	Isolated in Yamaguchi, Japan	This study	No PCR amplification	3
3401	+	Isolated in Yamaguchi, Japan	17	I	5
26695	+	Isolated in the United Kingdom	18	A	1
SS-1	+	Isolated in Australia	19	I	5
NCTC 11637	+	Isolated in USA	20	F	1
PY1	+	Hyogo, Japan	21	A	1
TK1402	-	Kanagawa, Japan	22	K	3

^a, "+" and "-" indicate that phage can and cannot form plaques, respectively.

^b, RFLP, restriction fragment length polymorphism.

^b, RFLP types are described in Fig. S1.

TABLE S2. List of *Helicobacter* spp. and phages used in the in silico analysis.

Organism	Genbank accession No.
<i>Helicobacter pylori</i> F16	AP011940
<i>Helicobacter pylori</i> F30	AP011941
<i>Helicobacter pylori</i> F32	AP011943
<i>Helicobacter pylori</i> F57	AP011945
<i>Helicobacter pylori</i> 35A	CP002096
<i>Helicobacter pylori</i> 51	CP000012
<i>Helicobacter pylori</i> 83	CP002605
<i>Helicobacter pylori</i> 52	CP001680
<i>Helicobacter pylori</i> v225d	CP001582
<i>Helicobacter pylori</i> Cuz20	CP002076
<i>Helicobacter pylori</i> PeCan4	CP002074
<i>Helicobacter pylori</i> Shi470	CP001072
<i>Helicobacter pylori</i> Sat464	CP002071
<i>Helicobacter pylori</i> 26695	AE000511
<i>Helicobacter pylori</i> B8	FN598874
<i>Helicobacter pylori</i> B38	FM991728
<i>Helicobacter pylori</i> G27	CP001173
<i>Helicobacter pylori</i> HPAG1	CP000241
<i>Helicobacter pylori</i> India7	CP002331
<i>Helicobacter pylori</i> P12	CP001217
<i>Helicobacter pylori</i> Lithuania75	CP002334
<i>Helicobacter pylori</i> SJM180	CP002073
<i>Helicobacter pylori</i> 2017	CP002571
<i>Helicobacter pylori</i> 2018	CP002572
<i>Helicobacter pylori</i> J99	AE001439
<i>Helicobacter pylori</i> 908	CP002184
<i>Helicobacter pylori</i> Gambia94/24	CP002332
<i>Helicobacter pylori</i> SouthAfrica7	CP002336
<i>Helicobacter felis</i> ATCC 49179	FQ670179
<i>Helicobacter hepaticus</i> ATCC 51449	AE017125
<i>Helicobacter bizzozeronii</i> CIII-1	FR871757
<i>Helicobacter mustelae</i> 12198	FN555004
<i>Helicobacter acinonychis</i> str. Sheeba	AM260522
<i>Helicobacter</i> phage KHP30	AB647160
<i>Helicobacter</i> phage KHP40	AB731695
<i>Helicobacter</i> phage 1961P	JQ617284
<i>Helicobacter</i> phage phiHP33	JF734911

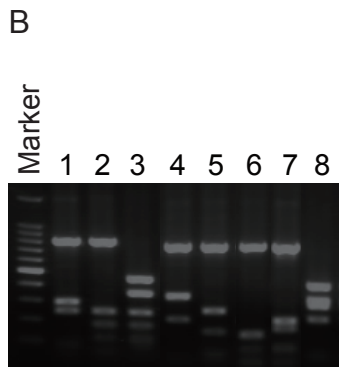
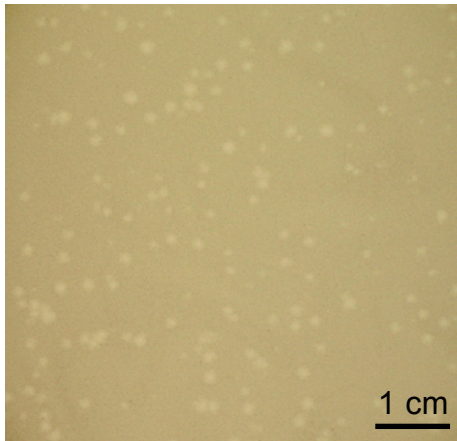


FIG. S1. Restriction fragment length polymorphism analysis of *H. pylori* strains used in this study. Representative restriction patterns of the restriction fragment length polymorphisms are shown. The 100 bp DNA ladder (Takara Bio) was electrophoresed in the leftmost lane of each panel, designated as "Marker". (A) Restriction patterns of UreAB digested by HaeIII. (B) Restriction patterns of FlaA digested by HhaI.

A



B

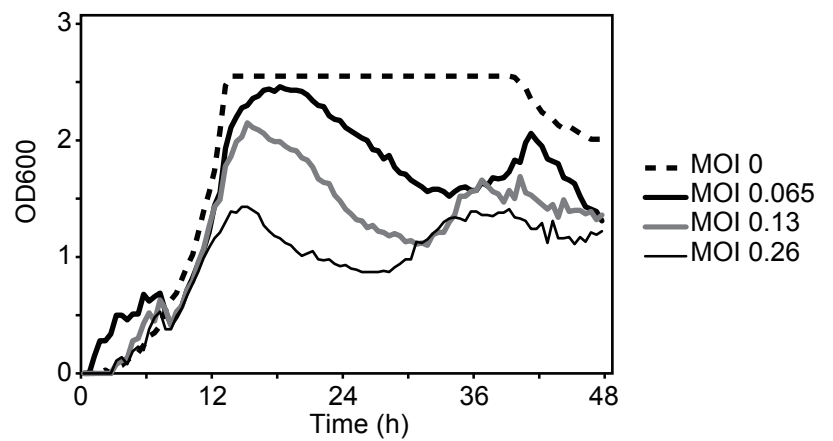


FIG. S2. (A) Photograph of phage plaques. Phage KHP30 was incubated with *H. pylori* strain 3401 for 2 days. (B) Lytic activity of phage KHP30 in liquid medium. *H. pylori* strain 3401 was added to BEV broth at a final concentration of 3.1×10^7 bacteria/ml. Phage KHP30 was added to the BEV medium containing *H. pylori* strain 3401 at MOIs of 0.065, 0.13, and 0.26. A no-phage culture was used as the control. The cultures (5 ml) were incubated in glass tubes with shaking, and bacterial growth was measured as the optical density at a wavelength of 600 nm using an automated turbidity meter (OD-Monitor C & T; Titec Co., Saitama, Japan).

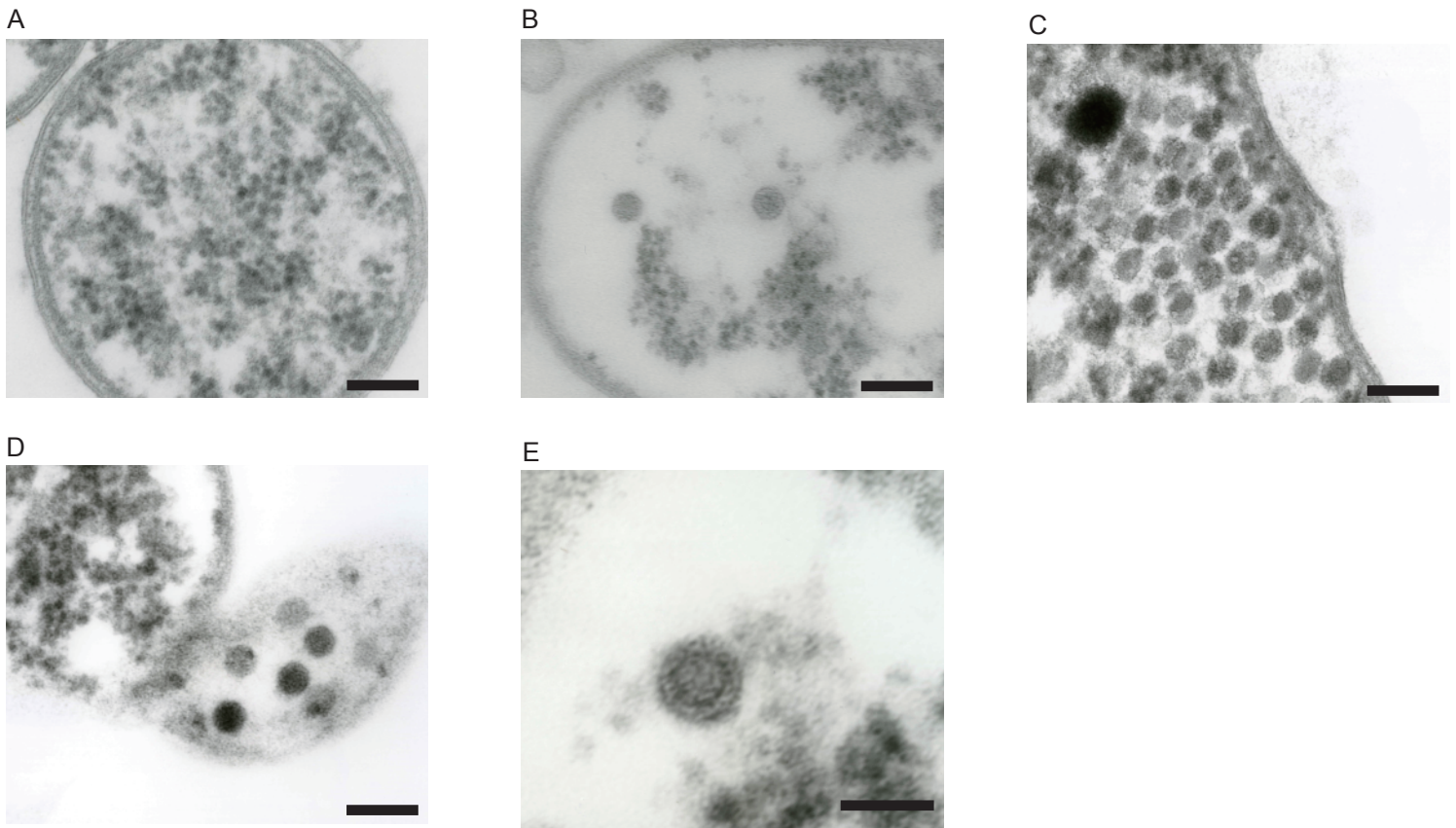


FIG. S3. Electron microscopic observation of thin sections of *H. pylori* and phage-infected *H. pylori*. (A) Thin section of *H. pylori* strain 3401. (B-E) Thin section of *H. pylori* strain 3401 infected with phage KHP30. *H. pylori* was infected with phage, and the thin sections were observed at B 140 min and C, D, E 280 min after phage infection. The observations were made with transmission electron microscopy. Phage particles were seen in B. The phage accumulated inside the cells, which are about to burst in C. Cell burst and phage release are shown in D. The possible layered structure of *H. pylori* phage KHP30 is visible in E. The bars indicate 100 nm in A–D and 50 nm in E.



FIG. S4. *In silico* analysis of potential integrated phages in the *H. pylori* genome. *H. pylori* whole genomes were analyzed with BLASTp against using GenomeMatcher, to identify sequences that could be aligned with the KHP30-like phage protein sequences. In each elongated rectangular panel, the vertical and horizontal axes indicate the *H. pylori* genome and the phage genome, respectively. The names of the *H. pylori* strains are listed on the top of the panel. The *H. pylori* strains were grouped into geographical types according to an *in silico* multiple-locus sequence analysis. Red circles, which contain similar ORF alignments to phage KHP30, indicate the possible integration of the phage. The bar on the right indicates the degree of similarity.

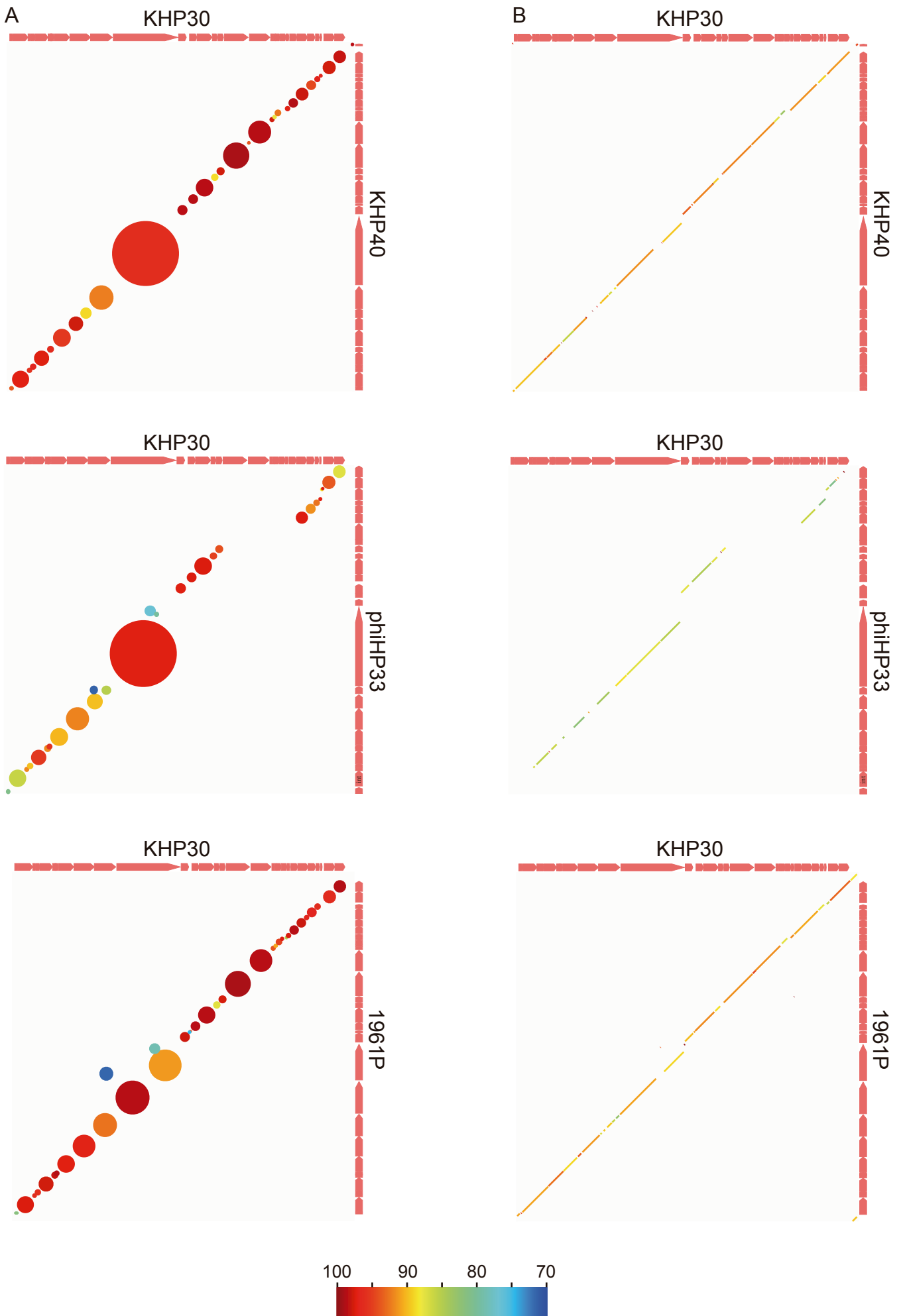


FIG. S5. BLAST analysis of phage KHP30 against the other *H. pylori* phages. *H. pylori* phages ϕ HP33, KHP40, and 1961P were used for this analysis. The analysis was performed with GenomeMatcher. In each panel, the horizontal and vertical axes indicate phage KHP30 and another *H. pylori* phage, respectively. The bar on the bottom indicates the degree of similarity. (A) BLASTp analysis. (B) BLASTn analysis. These phages are highly similar.

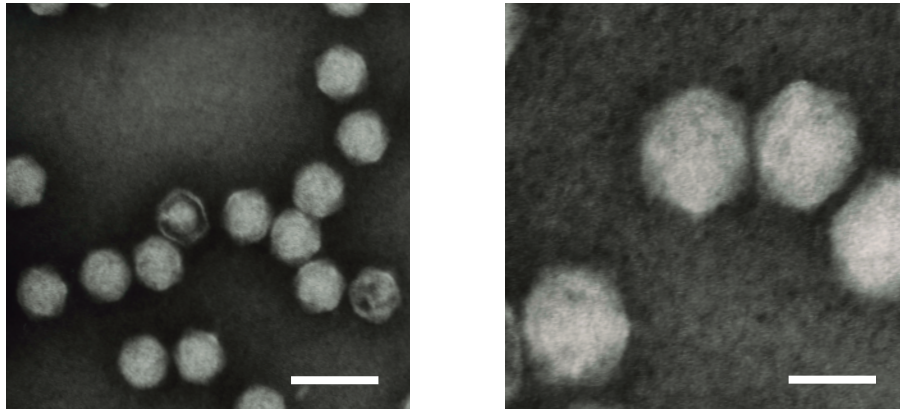


FIG. S6. Morphology of phage KHP40. Phage KHP40 was isolated from *H. pylori* strain KMT83, which was taken from a patient at Kochi University Hospital, Kochi, Japan. The purified phage was observed with transmission electron microscopy. The bars indicate 100 nm (left) and 50 nm (right). Phage KHP40 is a spherical phage with a diameter of 69.4 ± 2.3 nm (mean \pm SD; n = 11).