Supplementary Methods

One-step growth and phage adsorption rate tests: The one-step growth curve and phage adsorption rate tests for phage Bϕ–C62 were performed as described previously with some modifications (1, 2). To evaluate the one-step growth curve of phage Bϕ–C62, bacterial cells were cultured in 5 ml LB medium (OD_{600} = 0.2, 10^5 CFU/ml) for 2 h at 37℃ and added to phage solutions at a multiplicity of infection (MOI) of 0.001. After incubation for 5 min, free phages in the mixture were removed by centrifugation, and the pellet was resuspended in 5 ml fresh LB medium. Triplicate culture samples (100 µl) were collected at 5 min intervals for the first 30 min and at 10 min intervals for the next 100 min. Samples were immediately examined for phage titration.

To estimate the adsorption rate, host bacteria were grown in LB broth (OD_{600} = 0.5, 10^8 CFU/ml) for 4 h at 37℃ to the exponential phase and mixed with the phage solution at an MOI of 0.01. Mixed samples (100 µl) were collected at 1, 2, 3, 4, 5, and 10 min. After immediate centrifugation, the supernatant fluids were used to determine the titer of unabsorbed phages using the double layer agar plate method.

Thermal and pH stability tests: Evaluation of thermal and pH phage stability was performed as described previously with some modifications (3). Concentrated phage samples (10^9 PFU/ml) were incubated at the chosen temperature (RT, 37, 40, 50, 60, or 70℃) for 3, 6, or 9 h. Phage samples were also incubated at various pH levels (pH 4, 5, 6, 7, 7.5, 8, 9, and 10) for 1, 3, or 5 days and 1 and 10 months. The percentage of phage titer (PFU/ml) was compared to that of the control samples (4℃, pH 7.5).

Cytokine analysis: Cytokine concentration (TNF-α, IL-6) in lung lysates and serum samples was determined in duplicate using the commercial DuoSet ELISA kit (R&D Systems, MN, USA) according to the manufacturer’s recommendations.

Supplementary References

1. **Lin NT, Chiou PY, Chang KC, Chen LK, Lai MJ.** 2010. Isolation and characterization of phi AB2: a novel bacteriophage of Acinetobacter baumannii. Res Microbiol **161:**308-314.
2. **Karumidze N, Kusradze I, Rigvava S, Goderdzishvili M, Rajakumar K, Alavidze Z.** 2013. Isolation and characterisation of lytic bacteriophages of Klebsiella pneumoniae and Klebsiella oxytoca. Curr Microbiol **66:**251-258.
3. **Yang H, Liang L, Lin S, Jia S.** 2010. Isolation and characterization of a virulent bacteriophage AB1 of Acinetobacter baumannii. BMC Microbiol **10:**131.

Supplementary Figures and Table

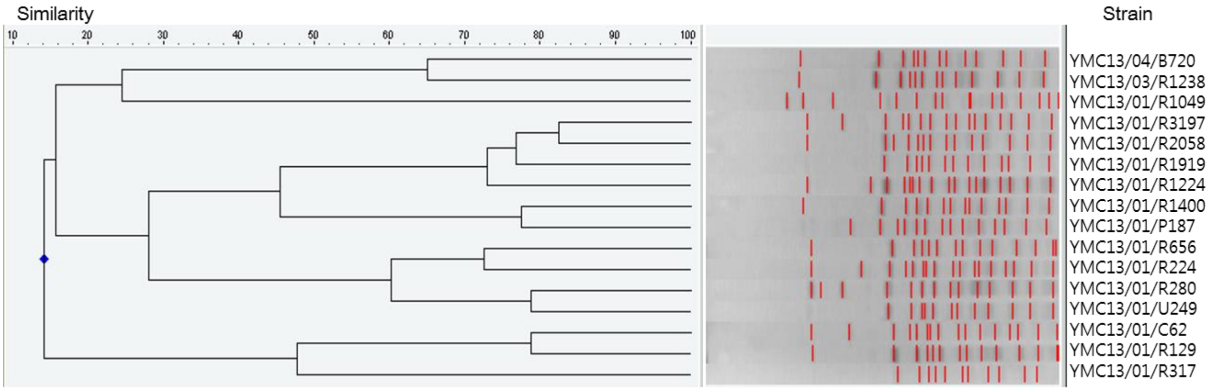


Fig. S1. PFGE pattern of *Xba*I-restricted chromosomal DNA from 16 clinical *A. baumannii* isolates. Each column indicates dendrogram analysis by InfoQuest FP software (version 4.50, Bio-Rad Laboratories, Inc), PFGE pattern using a CHEF-DR II system (Bio-Rad, Hercules, CA) and identical number of collected isolates. (electrophoresis conditions: 1% agarose; 6 V/cm; high, 10 s; low, 3 s; in a linear manner for 20 h). Percent similarity is indicated on the scale.

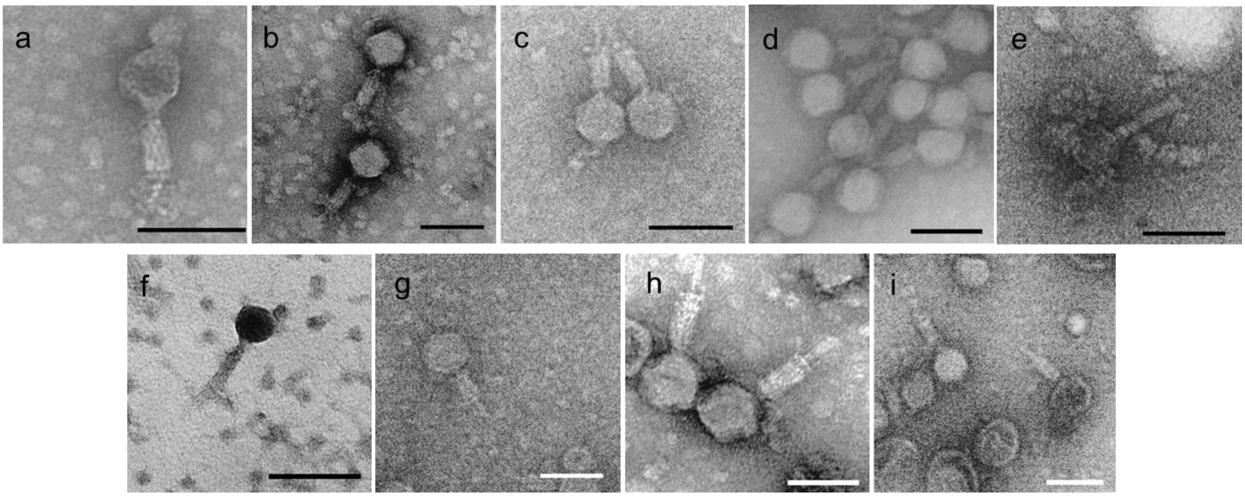


Fig. S2. Transmission electron microscopy images of 9 *A. baumannii* phages. (a) Bϕ-R129, (b) Bϕ-R430, (c) Bϕ-P447, (d) Bϕ-R1238, (e) Bϕ-R1520, (f) Bϕ-R2058, (g) Bϕ-R3128, (h) Bϕ-R3252, and (i) Bϕ-R3285. These *A. baumannii* phages belong to the *Myoviridae* family. Bar length of 100 nm.

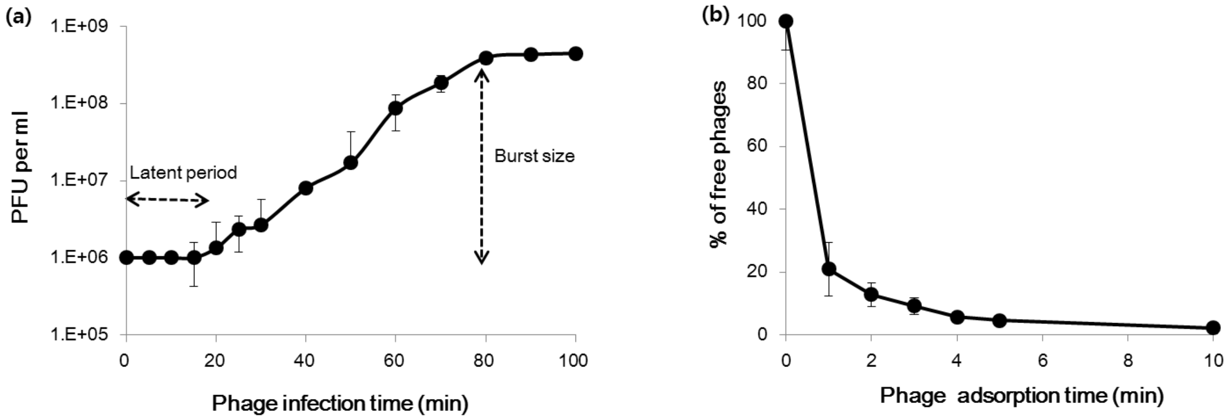


Fig. S3. One-step growth curve and adsorption rate of *A. baumannii* phage Bϕ-C62. One-step growth kinetics (a) and the adsorption rate (b) of phage Bϕ-C62 were evaluated using the *A. baumannii* YMC13/01/C62 host strain. The figure indicates alatent period of approximately 20 min and a burst size of 76 PFU/ml after 80 min (a) and shows that 80% of phages were adsorbed onto the host bacteria at 1 min (b). Each time point is the mean of three independent experiments, and the data are shown as the mean ± standard deviation.

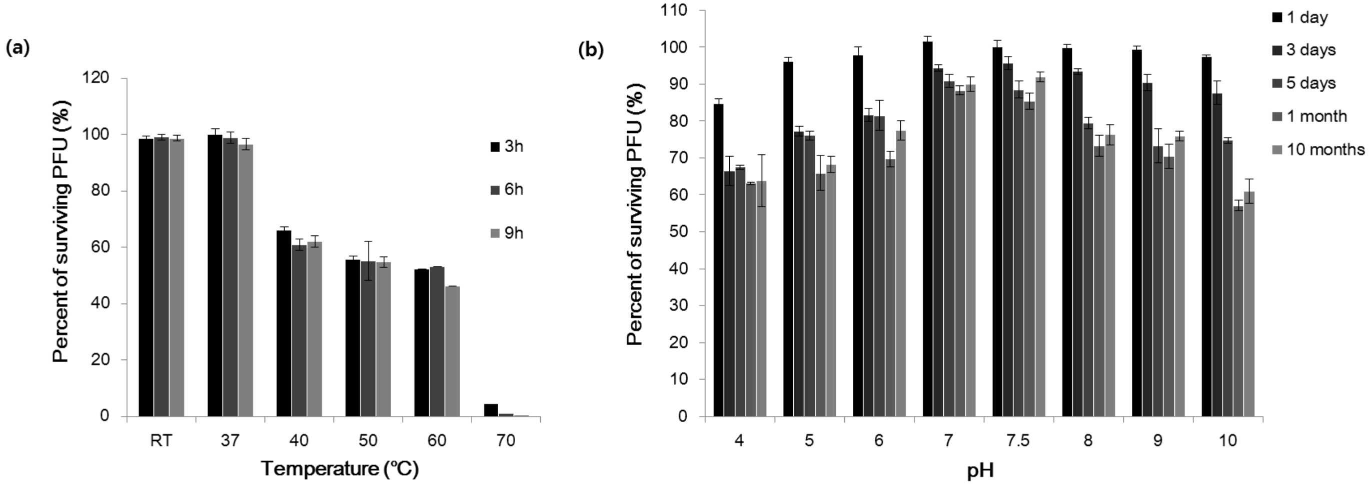
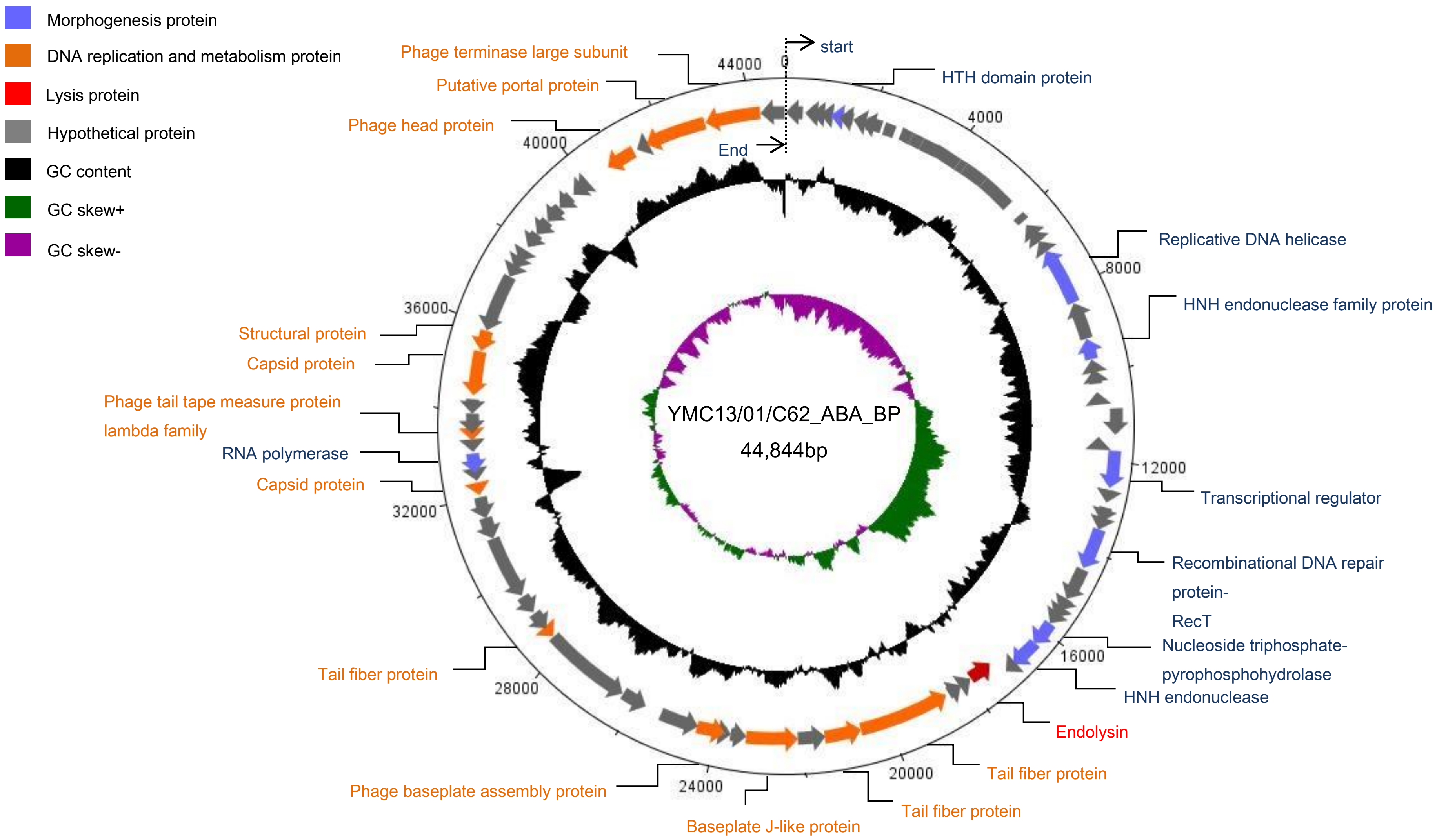


Fig. S4. The stability of *A. baumannii* phage Bϕ-C62 at various temperature and pH values. Phage were evaluated for stability kinetics at RT (25℃), 37℃, 40℃, 50℃, 60℃, and 70℃ for 9 h (a) and at different pH values (between pH 4 to pH 10) for 10 months (b) at the indicated time points. These values are represented as the percentage of surviving phages at each experimental point. Phage Bϕ-C62 exhibited high stability at RT and 37℃ for up to 9 h (a) and at pH 7 and 7.5 for10 months (b). The results shown are the mean ± standard deviation of triplicate experiments.



Morphogenesis protein
DNA replication and metabolism protein
Lysis protein
Hypothetical protein
GC content
GC skew+
GC skew-
start
End
0
4000
8000
12000
16000
20000
24000
28000
32000
36000
40000
44000
YMC13/01/C62_ABA_BP
44,844bp
HTH domain protein
Replicative DNA helicase
HNH endonuclease family protein
Transcriptional regulator
Recombinational DNA repair protein-RecT
Nucleoside triphosphate-pyrophosphohydrolase
HNH endonuclease
Endolysin
Tail fiber protein
Tail fiber protein
Baseplate J-like protein
Phage baseplate assembly protein
Tail fiber protein
Capsid protein
RNA polymerase
Phage tail tape measure protein lambda family
Capsid protein
Structural protein
Phage head protein
Putative portal protein
Phage terminase large subunit

Fig. S5. Circular map of the *A. baumannii* phage Bϕ-C62 genome prepared using DNAPlotter software. The putative ORFs along with transcription direction are represented by arrows, and the functional proteins, GC content, and GC skew are indicated by different colors.

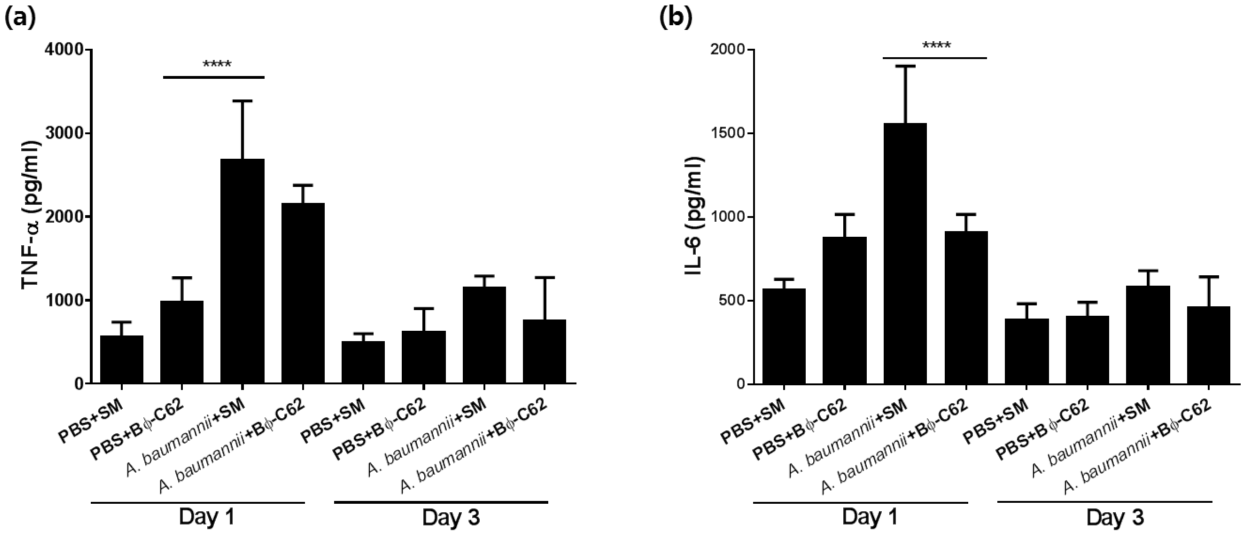


Fig. S6. Cytokine levels and histopathology changes in mouse lungs in intranasally (*A. baumannii* YMC13/01/C62)-infected mice or phage Bϕ-C62-treated mice. TNF-α (a) and IL-6 (b) levels were evaluated using ELISA. One-way ANOVA with Turkey`s multiple comparisons test was used to compare cytokine levels. Significant differences (*****p* < 0.0001) were observed at each time point.

Table S1. Antibiotic resistance profiles of *A. baumannii* strains used in this study[a] and host spectrum of *A. baumannii* phage Bϕ-C62

Host stain	Amikacin	Ampicillin/ Sulbactam	Ceftazidime	Colistin	Cefepime	Cefotaxime	Gentamicin	Imipenem	Levofloxacin	Meropenem	Minocycline	Piperacillin/ Tazobactam	Cotrimoxazole	Tigecycline	Infectivity of Bϕ-C62[b]
YMC13/02/R323	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤4 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R291	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/B1502	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/B1178	≤8 (S)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≤2 (S)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R931	≥64 (R)	≤8 (S)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≤4 (I)	≥16 (R)	≤4 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R401	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R426	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≤1 (S)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R427	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≤1 (S)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R464	≥64 (R)	≤8 (S)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≤4 (I)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/U501	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R319	≥64 (R)	≤8 (S)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/P148	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/P177	≥64 (R)	≤8 (S)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≥8 (R)	-
YMC13/02/B2199	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/P180	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/P335	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤8 (I)	≥128 (R)	≥320 (R)	≤2 (S)	-

[a], Antibiotic resistance was determined by the disk diffusion test method.

‘Susceptible’, ‘Intermediate’ and ‘Resistant’ are abbreviated as ‘S’, ‘I’ and ‘R’.

[b], Phage activity against collected bacteria: -, No plaque.

Table S1. (Continued) Antibiotic resistance profiles of *A. baumannii* strains used in this study[a] and host spectrum of *A. baumannii* phage Bϕ-C62

Host stain	Amikacin	Ampicillin/ Sulbactam	Ceftazidime	Colistin	Cefepime	Cefotaxime	Gentamicin	Imipenem	Levofloxacin	Meropenem	Minocycline	Piperacillin/ Tazobactam	Cotrimoxazole	Tigecycline	Infectivity of Bϕ-C62[b]
YMC13/02/B2343	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≥8 (R)	-
YMC13/02/R796	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R808	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≤4 (I)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R855	≤8 (S)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≤1 (S)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R869	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤1(S)	-
YMC13/02/R947	≥64 (R)	≤8 (S)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≤4 (I)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R972	≤8 (S)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2(S)	-
YMC13/02/R978	≤8 (S)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤2 (S)	≥128 (R)	≥320 (R)	≤1 (S)	-
YMC13/02/R1138	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/R990	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/U1368	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/U1431	≥64 (R)	≥32 (R)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≤2 (S)	-
YMC13/02/P267	≥64 (R)	≤16 (I)	≥64 (R)	≤0.5 (S)	≥64 (R)	≥64 (R)	≥16 (R)	≥16 (R)	≥8 (R)	≥16 (R)	≤1 (S)	≥128 (R)	≥320 (R)	≥8 (R)	-

[a], Antibiotic resistance was determined by the disk diffusion test method.

‘Susceptible’, ‘Intermediate’ and ‘Resistant’ are abbreviated as ‘S’, ‘I’ and ‘R’.

[b], Phage activity against collected bacteria: -, No plaque.

Table S2. *A. baumannii* phage Bϕ-C62 ORF summary.

Gene no.	**Range**		**Initiation codon**	**Strand**	**Length (bp)**	**Putative function**	**Annotation source**	**E-value**	**NCBI BlastP identity (%)**	**NCBI-Bank accession number**
	start	**stop**								
ORF1	5	394	ATG	-	390					
ORF2	448	615	ATG	-	168					
ORF3	602	808	ATG	-	207					
ORF4	798	1040	ATG	-	243					
ORF5	1037	1234	ATG	-	198	Binding HTH domain	[*Acinetobacter*phage IME-AB2]	4E-37	100	AFV51517.1
ORF6	1237	1563	ATG	-	327					
ORF7	1563	1778	GTG	-	216					
ORF8	1771	1893	ATG	-	123					
ORF9	1890	2237	ATG	-	348					
ORF10	2301	2540	ATG	-	240					
ORF11	2681	2962	GTG	-	282					
ORF12	2949	3209	ATG	-	261					
ORF13	3206	3586	ATG	-	381					
ORF14	3579	4160	ATG	-	582					
ORF15	4157	4321	GTG	-	165					
ORF16	4318	4893	ATG	-	576					
ORF17	4890	5645	ATG	-	756					
ORF18	5971	6156	ATG	-	186					
ORF19	6256	6432	ATG	-	177					
ORF20	6429	6722	ATG	-	294					
ORF21	6719	6985	ATG	-	267					
ORF22	6996	8339	ATG	-	1344	Replicative DNA helicase	[*Acinetobacter*phage IME-AB2]	0	100	AFV51534.1
ORF23	8345	9211	ATG	-	867					
ORF24	9204	9683	ATG	-	480	HNH endonuclease family protein	[*Acinetobacter*phage IME-AB2]	3E-112	100	AFV51536.1
ORF25	9696	9908	ATG	-	213					
ORF26	9923	10258	ATG	-	336					
ORF27	10442	10630	ATG	-	189					
ORF28	10824	11411	ATG	+	588					
ORF29	11464	11658	ATG	-	195					
ORF30	11758	12570	ATG	+	813	Transcriptional regulator	[*Acinetobacter* phage IME-AB2]	0	99	AFV51542.1
ORF31	12637	12894	ATG	+	258					
ORF32	12987	13319	ATG	+	333					
ORF33	13319	13501	ATG	+	183					
ORF34	13498	14397	ATG	+	900	Recombination DNA repair protein RecT	[*Acinetobacter*phage IME-AB2]	0	100	AFV51546.1
ORF35	14433	15149	ATG	+	717					
ORF36	15150	15443	ATG	+	294					
ORF37	15440	15631	ATG	+	192					

ORF38	15628	15789	ATG	+	162					
ORF39	15789	16349	ATG	+	561	Nucleoside triphosphate pyrophospho-hydrolase	[*Acinetobacter*phage IME-AB2]	6E-52	57	AFV51550.1
ORF40	16336	16977	ATG	+	642	HNH endonuclease	[*Staphylococcus* phage SA5]	3E-34	43	AFV80851.1
ORF41	16970	17200	GTG	+	231					
ORF42	17354	17908	ATG	-	555	Endolysin/ autolysin	[*Acinetobacter* bacteriophage AP22]	5E-130	98	YP_006383807.1
ORF43	17920	18144	ATG	-	225					
ORF44	18179	18514	GTG	-	336					
ORF45	18582	20678	ATG	-	2097	Tail fiber protein	[*Acinetobacter* phage IME-AB2]	5E-59	93	AFV51555.1
ORF46	20680	21510	GTG	-	831	Tail fiber protein	[*Acinetobacter* bacteriophage AP22]	1E-119	70	YP_006383803.1
ORF47	21503	22129	ATG	-	627					
ORF48	22129	23313	ATG	-	1185	Baseplate J-like protein	[*Acinetobacter* phage IME-AB2]	0	99	AFV51558.1
ORF49	23310	23663	ATG	-	354					
ORF50	23660	23806	ATG	-	147					
ORF51	23809	24456	ATG	-	648	Phage baseplate assembly protein	[*Acinetobacter* phage IME-AB2]	2E-154	99	AFV51561.1
ORF52	24437	25327	GTG	-	891					
ORF53	25716	26312	ATG	-	597					
ORF54	26320	28368	ATG	-	2049					
ORF55	28371	28613	GTG	-	243	Tail-fiber	[*Acinetobacter*phage IME-AB2]	0	92	AFV51565.1
ORF56	28613	29038	ATG	-	426					
ORF57	29084	29533	ATG	-	450					
ORF58	29546	31009	ATG	-	1464					
ORF59	30999	31493	ATG	-	495					
ORF60	31490	31993	ATG	-	504					
ORF61	32038	32298	ATG	-	261	Capsid protein	[*Acinetobacter*bacteriophage AP22]	0.16	33	YP_006383780.1
ORF62	32367	32552	ATG	-	186					
ORF63	32549	32986	ATG	-	438	RNA polymerase	[*Acinetobacter*phage IME-AB2]	2E-88	86	AFV51492.1
ORF64	33030	33215	ATG	-	186					
ORF65	33289	33435	ATG	-	147	Phage tail tape measure protein lambda family	[*Snodgrassellaalvi* wkB2]	0.13	61	AHN27888.1
ORF66	33451	33903	ATG	-	453					
ORF67	33903	34241	ATG	-	339					
ORF68	34321	35340	ATG	-	1020	Capsid protein	[*Acinetobacter* phage IME-AB2]	0	100	AFV51496.1
ORF69	35350	35829	ATG	-	480	Structural protein	[*Acinetobacter*phage IME-AB2]	3E-109	100	AFV51497.1
ORF70	35837	37171	ATG	-	1335					
ORF71	37171	37335	ATG	-	165					
ORF72	37385	37591	ATG	-	207					
ORF73	37581	37856	ATG	-	276					
ORF74	37955	38317	ATG	-	363					
ORF75	38314	38706	ATG	-	393					
ORF76	38699	39121	ATG	-	423					

ORF77	39111	39464	ATG	-	354					
ORF78	39546	39692	ATG	-	147					
ORF79	39693	39857	ATG	-	165					
ORF80	40547	41254	ATG	-	708	Phage head protein	[*Acinetobacter*phage IME-AB2]	1E-171	99	AFV51508.1
ORF81	41320	41577	ATG	-	258					
ORF82	41564	42997	ATG	-	1434	Portal protein	[*Acinetobacter*bacteriophage AP22]	0	90	YP_006383767.1
ORF83	43001	44278	ATG	-	1278	Phage terminase large subunit	[*Acinetobacte*r bacteriophage AP22]	0	84	YP_006383766.1
ORF84	44275	44829	TTG	-	555					