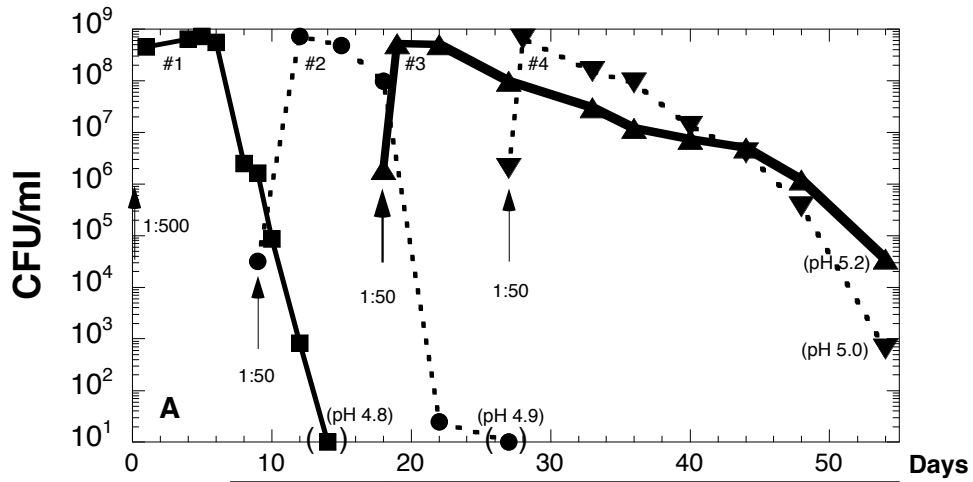


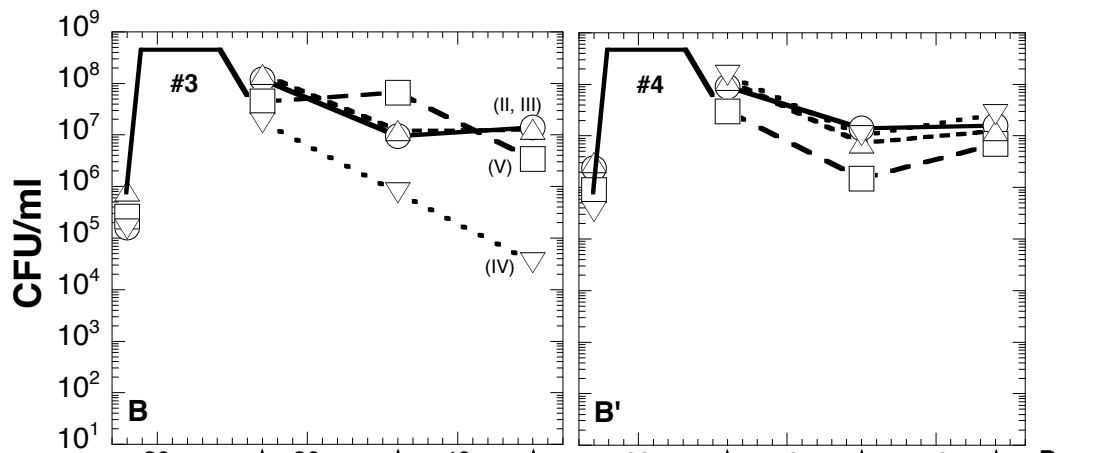
TABLE S1. *E. coli* K-12 strains

ENZ	Genotype	Source (reference)
535	F ⁻ <i>rph-1 ilvG rfb-50 phnE</i>	MG1655 (21)
985	As 535 but <i>rpoS359::Tn10</i>	(21)
1698	As 535 but <i>rpoS::kan</i>	(11)
1755	As 535 but $\Delta(\textit{phoB-R})\textit{kan}$	(21)
1791	As 535 but $\Delta\textit{lacY::kan}$	P1.JW334
1797	As 535 but <i>lacY::Tn10</i>	This study
1901	Evolved 535 RpoS ⁻	This study
1902	Evolved 535 Glg ⁻	This study
1903	Evolved 535 Glg ⁺	This study
1904	Evolved 535 Glg ⁺	This study
1905	Evolved 535 Glg ⁺	This study
1944	As 535 but $\Delta\textit{rpoS::kan}$	P1.JW5437
1946	As 1944 (535 $\Delta\textit{rpoS}$) Km ^s	This study
1984	As1905 but $\Delta\textit{lacY::kan}$	P1.JW334
2000	As 1901 but $\Delta\textit{lacY::kan}$	P1.JW334
2001	As 1902 but $\Delta\textit{lacY::kan}$	P1.JW334
2002	As 1903 but $\Delta\textit{lacY::kan}$	P1.JW334
2005	As 1944 (535 $\Delta\textit{rpoS::kan}$) but <i>cysC95::Tn10</i>	This study
2015	As 1901 but $\Delta(\textit{phoB-R})\textit{kan}$	P1.1755
2020	As 1946 (535 $\Delta\textit{rpoS}$) but $\Delta\textit{lacY::kan}$	P1.JW334
2039	As 535 but <i>rpoS819</i>	This study
2041	As 535 but <i>rpoS1901</i>	This study
2063	As 2039 (535 <i>rpoS819</i>) but $\Delta\textit{lacY::kan}$	P1.JW334
2065	As 2041 (535 <i>rpoS1901</i>) but $\Delta\textit{lacY::kan}$	P1.JW334
2075	As 535 but $\Delta\textit{phoB::kan}$	P1.JW389
2079	As 1901 but $\Delta\textit{phoB::kan}$	P1.JW389
2084	As 2075 (535 $\Delta\textit{phoB}$) Km ^s	This study
2255	As 535 but $\Delta\textit{proP::kan}$ MePn ⁻	This study
2256	As 535 but $\Delta\textit{proP::kan}$ MePn ⁺	This study
2258	As 1946 (535 $\Delta\textit{rpoS}$) but $\Delta\textit{proP::kan}$ MePn ⁻	This study
2259	As 1946 (535 $\Delta\textit{rpoS}$) but $\Delta\textit{proP::kan}$ MePn ⁺	This study
2260	As 1901 but $\Delta\textit{pstA::kan}$	P1.JW3704
2263	As 2041 (535 <i>rpoS1901</i>) but $\Delta\textit{proP::kan}$ MePn ⁺	This study
2265	As 2041 (535 <i>rpoS1901</i>) but $\Delta\textit{proP::kan}$ MePn ⁻	This study
2268	As 1901 but $\Delta\textit{phnK::kan}$ GPS ⁺ MePn ⁻	P1.JW5727
2271	As 1901 but $\Delta\textit{phnE::kan}$	P1.JW4065
2274	As 2271 (1901 $\Delta\textit{phnE::kan}$) Km ^s	This study
2277	As 2274 (1901 $\Delta\textit{phnE}$) but $\Delta\textit{pstA::kan}$	P1.JW3704
2278	As 2274 (1901 $\Delta\textit{phnE}$) but $\Delta\textit{phoE::kan}$	P1.JW231
2282	As 1901 but <i>lacY::Tn10</i>	P1.1797



(i)

RpoS-	2-d9	▲	▲	▲	▲	▲	▲	▲	▲	▲
Glg-	1	0	0	0	0	0	0	0	0	0
Glg+	5	0	0	0	0	0	0	0	0	0
RpoS-	3-d1	d4	d9	d15	d18	d22	d26	d30	d36	
Glg-	2	5	2	6	6	6	6	6	6	
Glg-	0	0	1	0	0	0	0	0	0	1
Glg+	4	1	3	0	0	0	0	0	0	0
RpoS-			4-d1	d6	d9	d13	d17	d21	d27	
Glg-			4	0	0	3	6	6	4	
Glg-			0	0	0	0	0	0	2	
Glg+			2	6	6	3	0	0	0	



(ii)	pH	6.8	6.8	6.9	6.8	6.8	6.8
	RpoS-	0	0	0	0	0	0
	Glg-	12	12	0	12	12	0
	Glg+	0	0	12	0	0	12
(iii)	pH	6.8	6.8	6.9	6.8	6.8	6.8
	RpoS-	0	0	0	0	0	0
	Glg-	12	10	0	12	8	0
	Glg+	0	2	12	0	4	12
(iv)	pH	5.3	5.3	5.3	6.8	6.8	6.8
	RpoS-	0	0	0	0	0	0
	Glg-	12	6	10	12	10	2
	Glg+	0	6	2	0	2	10
(v)	pH	5.7	6.8	6.9	5.6	5.4	6.8
	RpoS-	11	0	10	6	12	2
	Glg-	1	0	0	2	0	1
	Glg+	0	12	2	4	0	9

1904/05

1901
1902
1903

FIG. S1

FIG. S1. Adaptive evolution of *E. coli* K-12 under Pi starvation conditions. Strain ENZ535 was diluted 1:50 into fresh Pi-limiting medium every 9 days of incubation and incubated further for up to 36 days. Five independent experiments (I-V) are shown. (A) The values (CFU/ml) for experiment (I) are from Fig. 1. The numbers of colonies (total 6) exhibiting either an RpoS⁻, Glg⁻, or Glg⁺ phenotype are indicated below the figure. (B and B') For the experiments (II-V), the values for the subcultures #3 and #4 are shown in panels B and B', respectively. The pH of the spent media and the number of colonies (total 12) exhibiting either an RpoS⁻, Glg⁻, or Glg⁺ phenotype are indicated below the figures. Strains ENZ1901 (RpoS⁻), ENZ1902 (Glg⁻), ENZ1903 (Glg⁺) in experiment V, and strains ENZ1904 and ENZ1905 (Glg⁺) in experiments II were isolated on day 27 of incubation in subcultures #4.

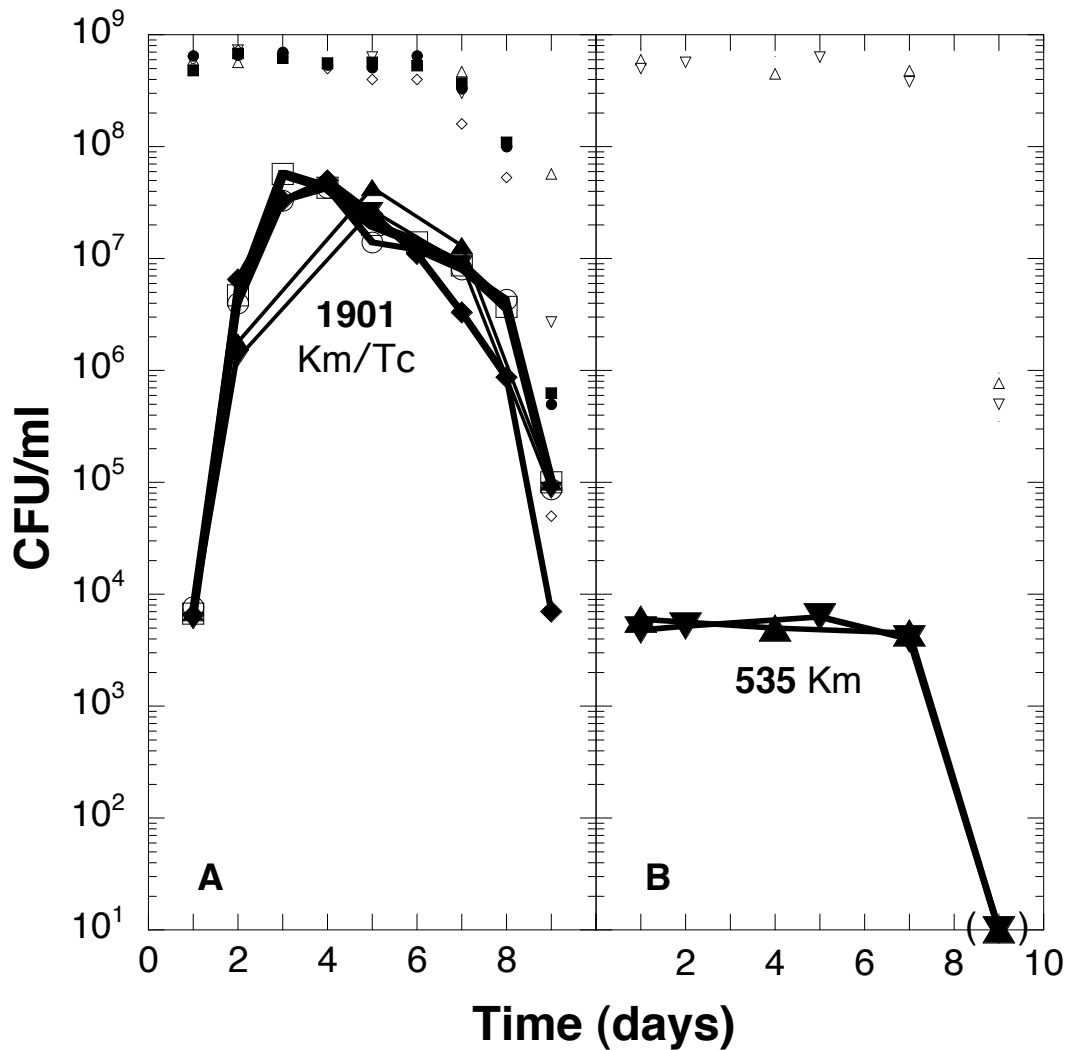


FIG. S2. The evolved strain ENZ1901 grows in a culture of *E. coli* K-12 starved for Pi. The strains tested as a minority in mixed cultures were grown as monocultures in Pi-limiting medium for 1 day, diluted 10^3 -fold, added (0.5 ml) into 50 ml of 1-day-old cultures of the ancestral strain ENZ535 in Pi-limiting medium, and incubated further for 8 days. (A) Evolved strain ENZ1901 (Km^r: ENZ2000) as a minority (▲, ▼, ◆) with ancestral strain ENZ535 (Tc^r: ENZ1797) as a majority (△, ▽, ◇), and evolved strain ENZ1901 (Tc^r: ENZ2282) as a minority (○, □) with ancestral strain ENZ535 (Km^r: ENZ1791) as a majority (●, ■). (B) Ancestral strain ENZ535 (Km^r: ENZ1791) as a minority (▲, ▼) with ancestral strain ENZ535 (Tc^r: ENZ1797) as a majority (△, ▽).

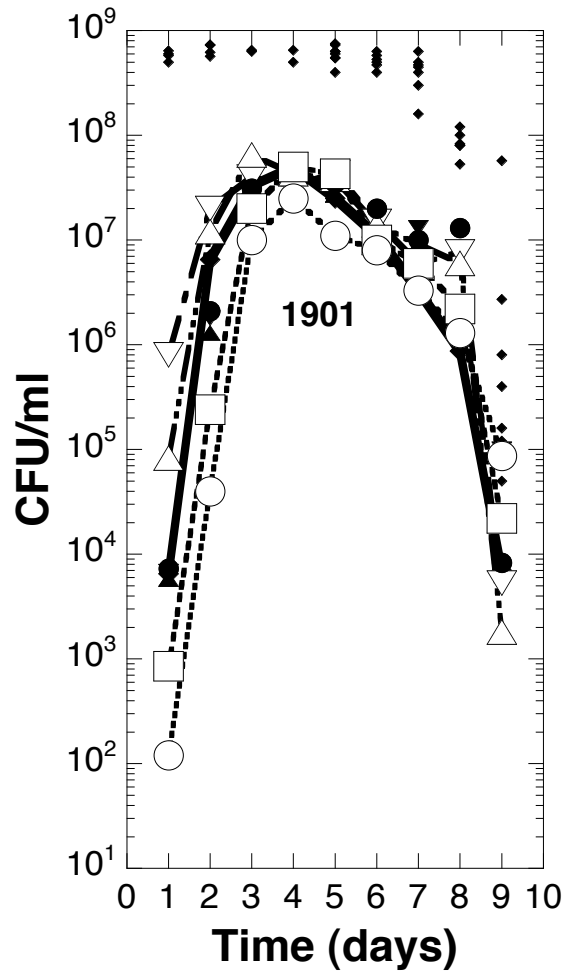


FIG. S3. Growth of the evolved strain ENZ1901 in mixed culture. The evolved strain ENZ1901 (Km^r : ENZ2000) was grown for 1 day in Pi-limiting medium, serially diluted (10^1 - 10^5 -fold) (∇ , \triangle , \bullet and \blacktriangle , \square , \circ), and 0.5 ml samples were added into 50 ml of 1-day-old cultures of the ancestral strain ENZ535 (Tc^r : ENZ1797) (\blacklozenge) in Pi-limiting medium, and incubated further for 8 days.

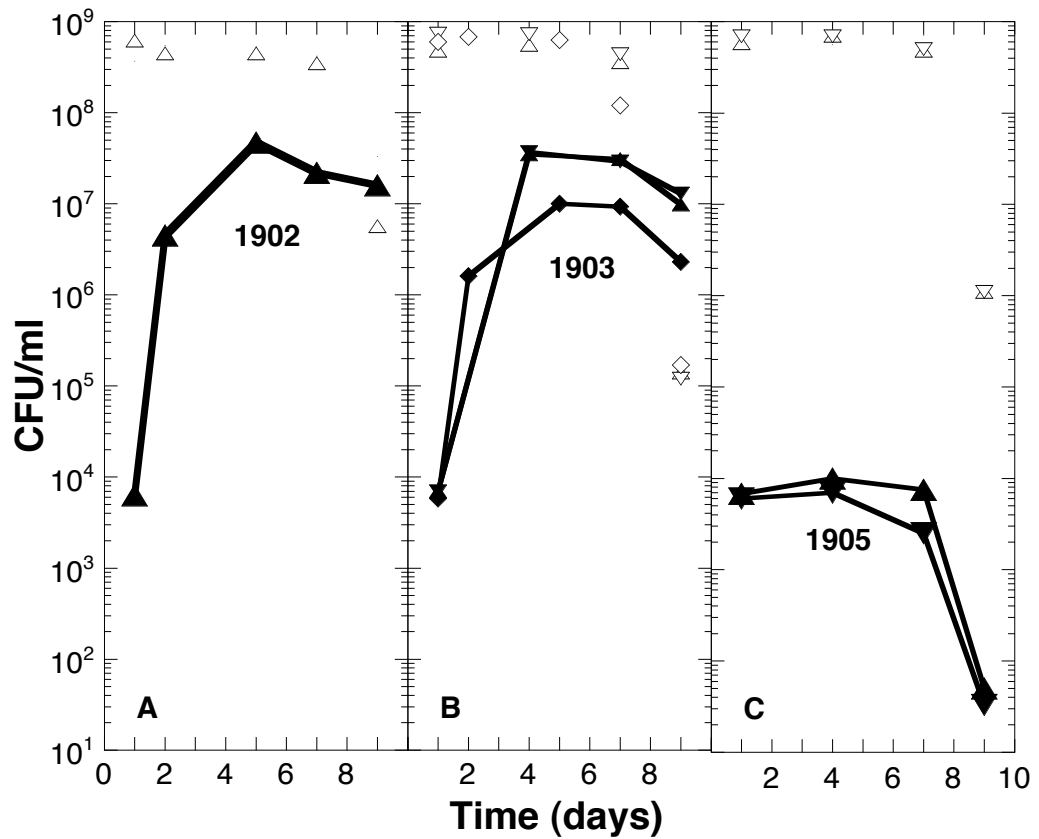


FIG. S4. Evolved strains in mixed culture. The evolved strains were grown as monocultures in Pi-limiting medium for 1 day, diluted 10³-fold, added (0.5 ml) into 50 ml of 1-day-old cultures of the ancestral strain ENZ535 (Tc^r: ENZ1797) (open symbols) in Pi-limiting medium, and incubated further for 8 days. (A) Evolved strain ENZ1902 (Glg⁻) (Km^r: ENZ2001) (▲). (B) Evolved strain ENZ1903 (Glg⁺) (Km^r: ENZ2002) (▲, ▼, ◆). (C) Evolved strain ENZ1905 (Glg⁺) (Km^r: ENZ1984) (▲, ▼).

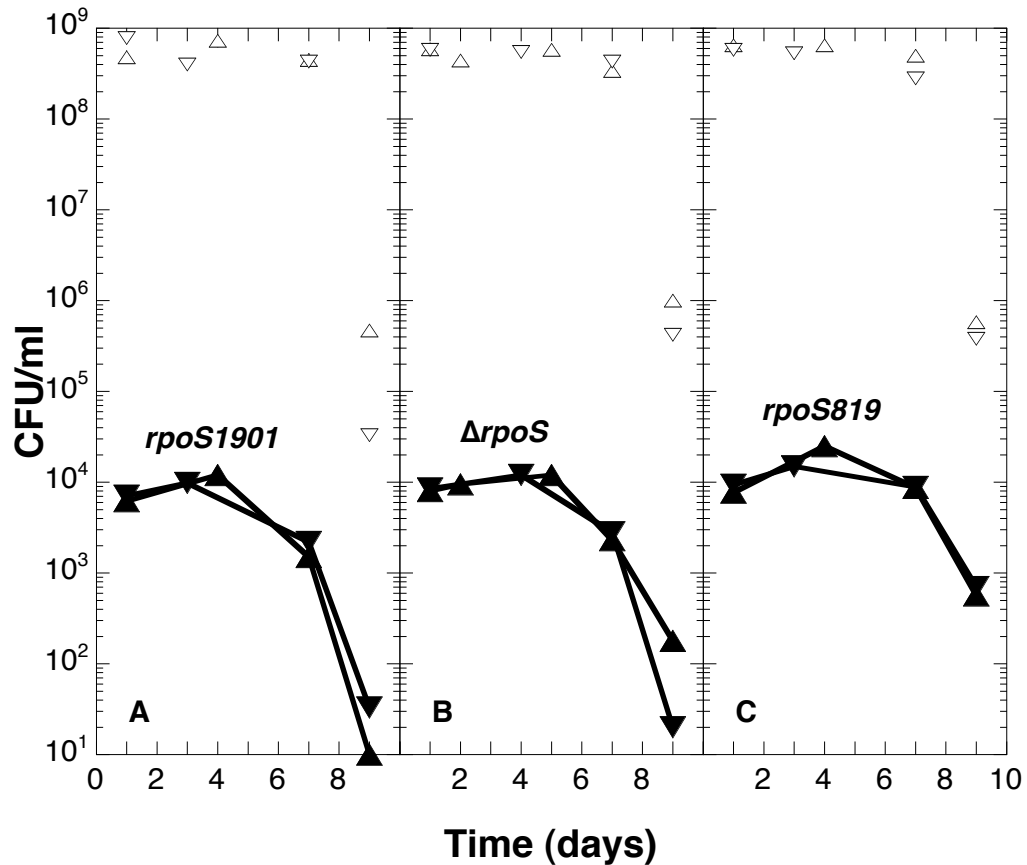


FIG. S5. ENZ535 carrying the *rpoS1901* allele alone does not grow in a mixed culture containing the ancestral strain ENZ535 in excess. The re-constructed strains (solid symbols) were grown as monocultures in Pi-limiting medium for 1 day, diluted 10³-fold, added (0.5 ml) into 50 ml of 1-day-old cultures of the ancestral strain ENZ535 (Tc^r: ENZ1797) in Pi-limiting medium (△, ▽), and incubated further for 8 days. (A) ENZ535 carrying the *rpoS1901* allele (Km^r: ENZ2065) (▲, ▼). (B) ENZ535 carrying the $\Delta rpoS$ allele (Km^r: ENZ2020) (▲, ▼). (C) ENZ535 carrying the *rpoS819* allele (Km^r: ENZ2063) (▲, ▼).

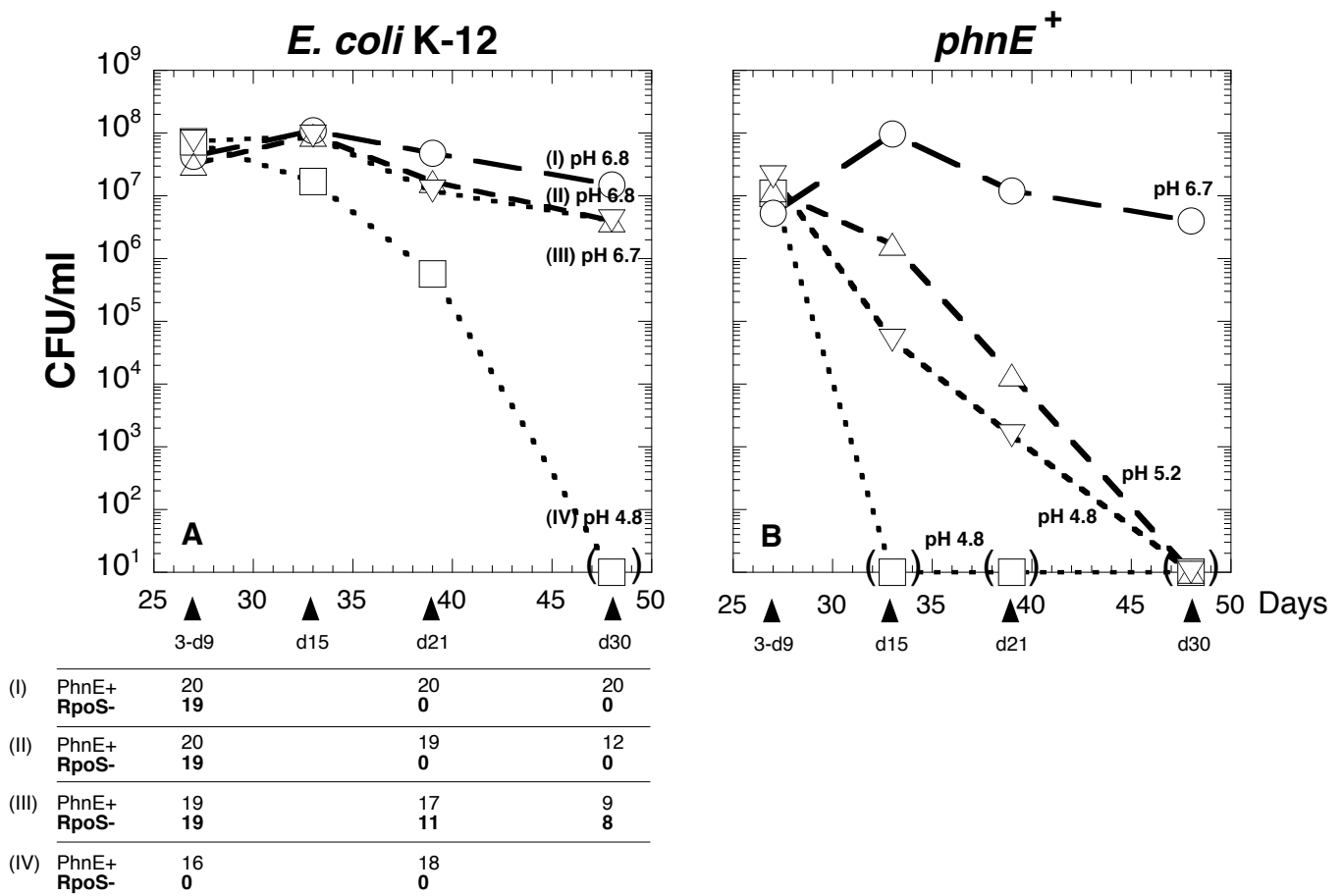


FIG. S6. Adaptive evolution of *E. coli* K-12 and of a *phnE*⁺ derivative. Strains were diluted 1:50 into Pi-limiting medium every 9 days of incubation and incubated further for 30 days; the values for the subcultures #3 are shown. The values for 4 independent experiments are shown for each strain. (A) *E. coli* K-12 (ENZ2255). The numbers of colonies (total 20) exhibiting a PhnE⁺ and/or an RpoS⁻ phenotype are indicated below the figure. (B) *E. coli* K-12 *phnE*⁺ (ENZ2256). The population that survived on day 30 of incubation (○) contained PhnE⁺ cells (20/20).