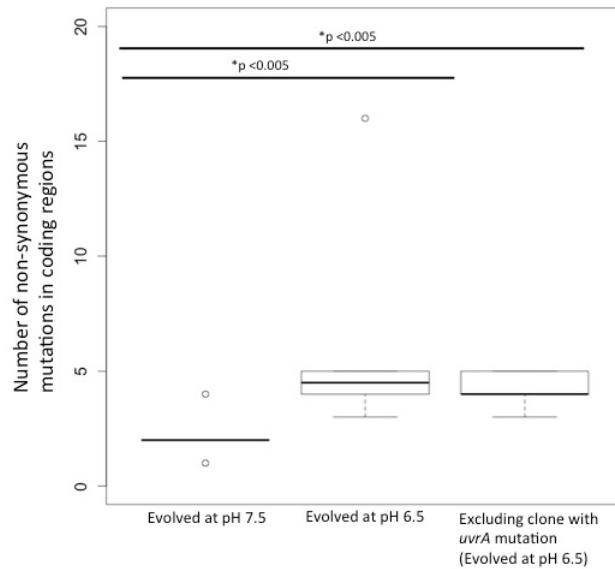
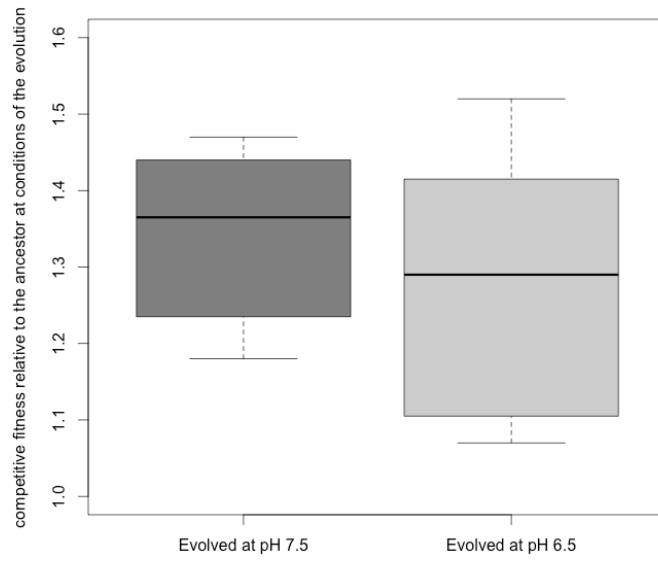


Supplementary Information

Supplementary Figures



Supplementary Figure S1. The number of non-synonymous mutations in coding regions in the evolved clones for each experimental evolution condition. Horizontal bars and P -values indicate the outcomes from two-sample Wilcoxon rank-sum tests. A star indicates a P -value < 0.05 . Data are presented as Tukey box-plots



Supplementary Figure S2. The relative fitness of the evolved clones calculated from competition with the ancestor at the conditions of the evolution with an initial frequency of 50% of evolved cultures. There is no significant difference in the increase in fitness between pH 7.5 and pH 6.5 (Wilcoxon rank sum test, $p > 0.4$, $n_1 = n_2 = 8$).

Supplementary Tables

Table S1. Mutations that accumulated in clones evolved at pH 7.5.

clone	position	mutation	annotation ^a	gene	description
C1	3,039,855	Δ3bp	coding (194-196/1296 nt)	<i>oprQ</i>	outer membrane protein OprE3
C2	1,418,905	A -> C	R401R (CGA -> CGC)	<i>fsr</i>	fosmidomycin resistance protein
C2	2,599,452	Δ12bp	coding (972-983/1293 nt)	<i>PST_2380</i>	porin
C2	2,799,697	+C	coding(486/777 nt)	<i>fliR</i>	flagellar biosynthesis protein FliR
C3	2,532,546	C -> T	noncoding (1207/2901 nt)	<i>PST_2318</i>	23S ribosomal RNA
C3	2,812,145	9bp x 2	duplication	<i>fliF</i>	flagellar MS-ring protein
C3	3,392,368	18bp x 2	duplication	<i>PST_3150</i>	hypothetical protein
C4	442,947	C -> A	D51Y (GAC -> TAC)	<i>phoP</i>	two-component response regulator PhoP
C4	3,392,467	C -> G	G278R (GGC -> CGC)	<i>PST_3150</i>	hypothetical protein
D1	1,485,794	Δ1 :: RP10 (-) 1+3bp :: +C	coding (227-229/1317 nt)	<i>flgE</i>	flagellar hook protein FlgE
D1	2,599,418	Δ27bp	coding (938-964/1293 nt)	<i>PST_2380</i>	porin
D1	3,361,859	Δ1 :: RP10 (-) 1+3bp :: +C	intergenic (+186/-26)	<i>PST_3122 / PST_3123</i>	hypothetical protein / general stress protein
D2	2,813,738	RP4 (+) +8np :: Δ1	Intergenic (-21/-75)	<i>fliE / PST_2592</i>	Flagellar hook-basal body protein FliE / benzoate transport protein
D2	3,040,582	6bp x 2	duplication	<i>oprQ</i>	outer membrane protein OprE3
D2	4,094,823	+G	coding (1241/1452 nt)	<i>PST_3786</i>	PAP2 family protein/DedA family protein
D3	442,505	C -> G	R198P (CGT -> CCT)	<i>phoP</i>	two-component response regulator PhoP
D3	1,484,866	Δ1 :: RP10 (-) + 3bp :: +C	coding (2-4/681 nt)	<i>flgD</i>	basal-body rod modification protein FlgD
D3	2,636,593	A -> G	R702R (CGA -> CGG)	<i>PST_2411</i>	assimilatory nitrate reductase
D3	3,392,355	+TGA	coding (944/1005 nt)	<i>PST_3150</i>	hypothetical protein
D3	4,283,751	Δ84bp	coding (3623-3706/7335 nt)	<i>PST_3948</i>	component of chemotactic signal transduction system
D4	1,484,866	Δ1 :: RP10 (-) + 3bp :: +C	coding (2-4/681 nt)	<i>flgD</i>	basal-body rod modification protein FlgD
D4	3,392,355	+TGA	coding (944/1005 nt)	<i>PST_3150</i>	hypothetical protein

Definitions: C clones, clones evolved from the ancestral strain 1601ech. D clones, clones evolved from the ancestral strain 1601gfp.

^aThe type of change that the mutation has caused. D51Y (GAC -> TAC) means that a base pair substitution from G to a T has caused amino acid 51 in this protein to change from aspartic acid (D) to tyrosine (Y). * signifies a stop codon. Intergenic (+186/-26) means that this mutation is located 186 base pairs downstream of one gene and 26 base pairs upstream of another. Coding (972-983/1293 nt) means the mutation is in (between) base pairs 972-983 out of 1293 nucleotides in the coding region of a protein.

Table S2. Mutations that accumulated in clones evolved at pH 6.5.

clone	position	mutation	annotation ^a	gene	description
G1	993,847	G -> A	L178F (CTC -> TTC)	<i>narX</i>	two-component sensor NarX
G1	1,260,110	T -> G	T411P (ACC -> CCC)	<i>pykA</i>	pyruvate kinase
G1	2,493,376	A -> C	H104P (CAC -> CCC)	<i>PST_2283</i>	outer membrane protein
G1	2,599,421	Δ12bp	coding (941-952/1293 nt)	<i>PST_2380</i>	porin
G1	2,809,584	C -> T	G78D (GGC -> GGC)	<i>fliI</i>	flagellum-specific ATP

			GAC		synthase
G2	754,814	+G :: RP10 (+) +3bp :: Δ1	intergenic (+45/-407)	<i>PST_0656 / PST_0657</i>	hypothetical protein / transposase like protein TbpA3
G2	1,260,604	A -> G	V246A (GTG -> GCG)	<i>pykA</i>	pyruvate kinase
G2	2,532,546	C -> T	Noncoding (1207/2901 nt)	<i>PST_2318</i>	23S ribosomal RNA
G2	3,040,113	T -> C	L151P (CTG -> CCG)	<i>oprQ</i>	outer membrane protein OprE3
G2	3,209,034	T -> G	T53P (ACC -> CCC)	<i>PST_2974</i>	3-hydroxyisobutyrate dehydrogenase
G2	2,666,603	C -> T	V801 (GTA -> ATA)	<i>pilF</i>	Type IV pilus biogenesis protein pilF
G2	3,818,939	Δ1bp	intergenic (- 17/+53)	<i>nirT / nirS</i>	tetraheme protein nirT / cytochrome cd1 nitrite reductase
G3	379,263	G -> A	G51D (GGC -> GAC)	<i>fbp</i>	fructose- 1,6 - biphosphatase
G3	442,411	Δ408bp		<i>phoP - phoQ</i>	
G3	2,415,795	T -> C	intergenic (- 1849/-67)	<i>PST_2210 / PST2213</i>	udecaprenyl pyrophosphatase / hypothetical protein
G3	2,771,593	T -> C	D338G (GAC -> GGC)	<i>fleQ</i>	transcriptional regulator FleQ
G3	3,040,113	T -> C	L151P (CTG -> CCG)	<i>oprQ</i>	outer membrane protein OprE3
G3	4,056,643 4,057,686	RP3 (+) Δ1bp Δ66bp	intergenic (+170/+2139) intergenic (+1213/+1029)	<i>PST_3751 / PST_3752</i>	CRISPR-associated Cas2 family protein / hypothetical protein
G4	379,170	C -> G	P20R (CCG -> CGG)	<i>fbp</i>	fructose- 1,6 - bisphosphatase
G4	995,501	G -> C	G286R (GGC -> CGC)	<i>narK</i>	nitrite extrusion protein
G4	2,532,535 2,532,546 2,532,548	G -> C C -> T C -> G	noncoding (1218/2901 nt) (1207/2901 nt) (1205/2901 nt)	<i>PST_2318</i>	23S ribosomal RNA
G4	3,040,113	T -> C	L151P (CTG -> CCG)	<i>oprQ</i>	outer membrane OprE3
H1	442,210	C -> G	A93P (GCC-> CCC)	<i>phoQ</i>	two-component sensor PhoQ
H1	881,077	C -> T	A432V (GCG -> GTG)	<i>rpoB</i>	DNA-directed RNA polymerase subunit beta
H1	1,926,532	G -> T	S508Y (TCC -> TAC)	<i>xcpQ</i>	general secretion pathway protein D
H1	2,796,711	G -> A	Q223* (CAG - >TAG)	<i>flhA</i>	flagellar biosynthesis protein FlhA
H1	2,875,014	G -> A	P24L (CCG -> CTG)	<i>gap-2</i>	glyceraldehyde-3- phosphate dehydrogenase
H1	4,057,221	Δ67bp	intergenic (+748/+1493)	<i>PST_3751 / PST_3752</i>	CRISPR-associated Cas2 family protein / hypothetical protein
H2	17,482	A -> G	V195A (GTC -> GCC)	<i>PST_0015</i>	amino acid ABC transporter permease
H2	197,672	G -> A	A64T (GCC -> ACC)	<i>ohr</i>	organic hydroperoxide resistance protein
H2	284,771	C -> T	G508D (GGT -> GAT)	<i>PST_0249</i>	hypothetical protein
H2	318,618	C -> T	L353L (CTG -> TTG)	<i>argA</i>	N-acetylglutamate synthase
H2	379,637	C -> T	L176F (CTT -> TTT)	<i>fbp</i>	fructose-1,6- bisphosphatase
H2	712,930	G -> A	P8S (CCA -> TCA)	<i>PST_0627</i>	hypothetical protein
H2	810,646	RP6(-)+7bp	intergenic (+55/+33)	<i>PST_0704 / PST_0705</i>	hypothetical protein / hypothetical protein
H2	850,146	C -> T	G96G (GGC -> GGT)	<i>gph</i>	phosphoglycolate phosphatase

H2	876,102	C -> T	intergenic (+9/-43)	<i>tuf / PST_0770</i>	elongation factor Tu / tRNA-Trp
H2	910,678	RP6 (+) +7bp	coding (364-370/2958nt)	<i>uvrA</i>	excinuclease ABC subunit A
H2	1,260,392	C -> T	A317T (GCG -> ACG)	<i>pykA</i>	pyruvate kinase
H2	1,510,239	RP6(-)+6bp	coding (726-731/1773nt)	<i>PST_1412</i>	EAL/GGDEF domain-containing protein
H2	1,575,460	G -> A	K89K (AAG -> AAA)	<i>PST_1469</i>	type I restriction-modification system, M subunit
H2	1,823,732	G -> A	L383L (CTG -> CTA)	<i>PST_1694</i>	acyl-CoA dehydrogenase
H2	1,948,354	Δ1bp	coding (600/1440nt)	<i>cydA2</i>	cytochrome d ubiquinol oxidase, subunit I
H2	2,332,524	G -> A	R259Q (CGG -> CAG)	<i>PST_2148</i>	hypothetical protein
H2	2,404,122	C -> T	C3C (TGC -> TGT)	<i>PST_2202</i>	hypothetical protein
H2	2,532,546	C -> T	noncoding (1207/2901nt)	<i>PST_2318</i>	23S ribosomal RNA
H2	2,812,452	T -> C	E299G (GAG -> GGG)	<i>fliF</i>	flagellar MS-ring protein
H2	3,039,855	Δ3bp	coding (194-196/1296nt)	<i>oprQ</i>	outer membrane protein OprE3
H2	3,086,463	G -> A	F89F (TTC -> TTT)	<i>PST_2869</i>	gamma-glutamyltranspeptidase
H2	3,495,820	G -> A	R423C (CGC -> TGC)	<i>PST_3235</i>	alpha, alpha-trehalose-phosphate synthase
H2	3,590,596	C -> T	G131D (GGT -> GAT)	<i>folP</i>	dihydropteroate synthase
H2	3,937,070	T -> C	D282G (GAC -> GGC)	<i>PST_3647</i>	hypothetical protein
H2	3,942,203	C -> T	R281R (CGG -> CGA)	<i>PST_3650</i>	hypothetical protein
H2	4,391,436	C -> T	S34F (TCT -> TTT)	<i>tctD</i>	transcriptional regulatory protein TctD
H3	379,227	A -> G	H39R (CAC -> CGC)	<i>fbp</i>	fructose-1,6-bisphosphatase
H3	442,534	G -> C	N188K (AAC -> AAG)	<i>phoP</i>	two-component response regulator PhoP
H3	2,772,535	Δ1bp	coding (71/1515nt)	<i>fleQ</i>	transcriptional regulator FleQ
H3	3,040,113	T -> C	L151P (CTG -> CCG)	<i>oprQ</i>	outer membrane protein OprE3
H4	379,374	C -> T	A88V (GCT -> GTT)	<i>fbp</i>	fructose-1,6-bisphosphatase
H4	2,771,479	T -> C	N376S (AAC -> AGC)	<i>fleQ</i>	transcriptional regulator FleQ
H4	3,040,113	T -> C	L151P (CTG -> CCG)	<i>oprQ</i>	outer membrane protein OprE3
H4	3,993,470	G -> A	P668S (CCG -> TCG)	<i>PST_3693</i>	tetrathionate reductase subunit A
H4	4,231,226	T -> C	I18V (ATT -> GTT)	<i>PST_3901</i>	hypothetical protein

Definitions: G clones, clones evolved from the ancestral strain 1601ech. H clones, clones evolved from the ancestral strain 1601gfp.

^aThe type of change that the mutation has caused. L178F (CTC -> TTC) means that a base pair substitution from C to a T has caused amino acid 178 in this protein to change from leucine (L) to phenylalanine (F). * signifies a stop codon. Intergenic (+45/-407) means that this mutation is located 45 base pairs downstream of one gene and 407 base pairs upstream of another. Coding (941-952/1293 nt) means the mutation is in (between) base pairs 941-952 out of 1293 nucleotides in the coding region of a protein

Table S3. Strains and plasmids.

Strain or plasmid	Relevant characteristics	Reference or source
<i>P. stutzeri</i> strain		
A1601gfp	A1501 with $\Delta comA$ and mini-Tn7T-LAC-Gm- <i>egfp</i> ; Gm ^R , <i>egfp</i> ⁺	This study
A1601ech	A1501 with $\Delta comA$ and mini-Tn7T-LAC-Gm- <i>echerry</i> ; Gm ^R , <i>echerry</i> ⁺	This study
<i>E. coli</i> strain		
DH5 α /λpir	Used for replication of pUC18T and pAW19 derivatives; λpir80 <i>dlacZ</i> Δ <i>M15</i> Δ(<i>lacZYA-argG</i>) <i>U169</i> <i>recA1</i> <i>hsdr17</i> <i>deoR</i> <i>thi-1</i> <i>supE44</i> <i>gyrA96</i> <i>relA</i>	(Miller and Mekalanos, 1988)
SM10/λpir	Used for replication of pUX-BF13; <i>thi-1</i> <i>thr</i> <i>leu</i> <i>tonA</i> <i>lacY</i> <i>supE</i> <i>recA</i> ::RP4-2-Tc::Mu, Km ^R , λpir	(Miller and Mekalanos, 1988)
BW20767	Used for conjugative transfer of pAW19 derivatives; RP4-2-Tc::Mu-1 Kan::Tn7 integrant <i>leu-63</i> ::IS10 <i>recA1</i> <i>zbf-5</i> <i>creB510</i> <i>hsdR17</i> <i>endA1</i> <i>thi</i> <i>uidA</i> (Δ <i>MluI</i>)::pir ⁺	(Metcalf <i>et al.</i> , 1995)
Plasmid		
pUC18T-mini-Tn7T-LAC-Gm	pUC18-based conditionally replicative delivery plasmid for mini-Tn7-LAC-Gm; Ap ^R , Gm ^R , mob ⁺	(Choi <i>et al.</i> , 2005)
pUC18T-mini-Tn7T-LAC-Gm- <i>egfp</i>	pUC18T-mini-Tn7T-LAC-Gm containing <i>egfp</i> immediately downstream of P _{lac} ; Ap ^R , Gm ^R , mob ⁺ , <i>egfp</i> ⁺	This study
pUC18T-mini-Tn7T-LAC-Gm- <i>echerry</i>	pUC18T-mini-Tn7T-LAC-Gm containing <i>echerry</i> immediately downstream of P _{lac} ; Ap ^R , Gm ^R , mob ⁺ , <i>echerry</i> ⁺	This study
pUX-BF13	R6K replicon-based helper plasmid that provides the Tn7T transposition function in <i>trans</i> ; Ap ^R , mob ⁺	(Bao <i>et al.</i> , 1991)

Table S4. Oligonucleotide PCR primers used for cloning the egfp or echerry gene into the pUC18T-mini-Zn7T-LAC-Gm plasmid.

Gene	Direction	^a Primer sequence (5'-3')	Restriction site
<i>egfp</i>	Forward	CGCGGATCCTGATTAACTTTATAAGGAGGAAAAAC ATATGAGTAAAGGAGAAGAAGTTTCACT	BamHI
	Reverse	CGGGGTACCTTTGTATAGTTCATCCATGCCATG	KpnI
<i>echerry</i>	Forward	CGCGGATCCTGATTAACTTTATAAGGAGGAAAAAC ATATGGTTTCCAAGGGCGAG	BamHI
	Reverse	CGGGGTACCTTATTTGTACAGCTCATCCATGC	KpnI

^aRed: leader sequence for restriction digestion. Blue: restriction sequence. Green: ribosomal binding sequence. Black: target-specific sequence.

