

Table S1 Single-nucleotide polymorphisms in evolved WT

Position	Annotation	Referece base	Altered base	Altered amino acid	Frequency																	
					R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT	R1_FT	R1_AT		
1527188	<i>from_yncH_ to_ydcD</i>	T	C	-	0.99	1.00	1.00	1.00	0.98	0.96	1.00	1.00	1.00	0.99	0.95	1.00	0.99	1.00	-	-	-	-
3214380	<i>rpoD</i>	A	C	Asp445Ala	0.99	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-
3214755	<i>rpoD</i>	A	G	Asp570Gly	0.93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1875676	<i>ninT</i>	A	C	Thr2Pro	0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3998195	<i>uvrD</i>	T	G	His72fs	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
302463	<i>paoA</i>	C	G	Ala37Ala	0.34	-	-	-	-	-	0.29	0.33	-	-	-	-	-	0.38	0.36	-	-	0.33
2718321	<i>yfiF</i>	G	C	Arg70Gly	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2074685	<i>from_flu_ to_yeeR</i>	G	C	-	0.31	-	0.34	-	-	-	0.33	-	-	-	0.35	-	-	-	-	-	-	-
707976	<i>from_glnS_ to_chiZ</i>	T	G	-	0.19	0.28	-	0.27	0.22	0.33	-	-	-	-	-	0.25	0.27	0.20	-	0.20	-	0.28
1381291	<i>from_ycjU_ to_ompG</i>	A	T	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2736931	<i>from_bamD_ to_raiA</i>	A	T	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3194593	<i>from_yqiK_ to_sibD</i>	A	G	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3440212	<i>rpoA</i>	T	C	Glu273Gly	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2800855	<i>nrdH</i>	G	A	Ala45Thr	-	-	0.99	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3214380	<i>rpoD</i>	A	T	Asp445Val	-	-	0.99	1.00	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-
3400123	<i>mreB</i>	A	C	Leu322Arg	-	-	0.98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3535354	<i>envZ</i>	A	C	Ile172Arg	-	-	0.97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4092667	<i>frvA</i>	A	G	Ser53Pro	-	-	0.91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4092664	<i>frvA</i>	T	C	Thr54Ala	-	-	0.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4092660	<i>frvA</i>	C	CGCGT	Gly55fs	-	-	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4095852	<i>rhaA</i>	T	C	Asp44Gly	-	-	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3233718	<i>from_fadH_ to_higA</i>	G	C	-	-	-	0.42	0.37	-	0.39	-	-	0.48	-	-	-	-	-	0.57	-	0.36	-
3491701	<i>from_yhfG_ to_ppiA</i>	C	G	-	-	-	0.38	-	0.42	-	0.28	-	-	0.28	0.29	-	-	-	-	0.33	0.32	-
2312798	<i>from_ompC_ to_micF</i>	C	CAGGTG	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2204829	<i>yehL</i>	T	G	Gly78Gly	-	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4094134	<i>rhaD</i>	C	T	Asp47Asn	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4184040	<i>rpoB</i>	GGTCTTTACTCGC	G	Val933 Arg936del	-	-	-	-	0.98	-	-	-	-	-	-	-	-	-	-	-	-	-
1799271	<i>from_pheM_ to_rplT</i>	T	C	-	-	-	-	0.97	0.99	-	-	-	-	-	-	-	-	-	-	-	-	-
720884	<i>from_speFL_ to_kdpE</i>	A	G	-	-	-	-	0.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2003387	<i>fliC</i>	C	A	Val74Phe	-	-	-	-	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-
4531697	<i>from_sgcX_ to_yjhP</i>	T	C	-	-	-	-	0.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4531698	<i>from_sgcX_ to_yjhP</i>	A	C	-	-	-	-	0.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4082690	<i>fdoH</i>	G	C	Arg24Gly	-	-	-	-	0.31	-	-	-	-	-	-	-	-	-	-	-	-	-

3623441	<i>yhhH</i>	A	ACC	Arg15fs	-	-	-	-	0.29	-	-	-	-	-	-	-	-	-	-	-	-	-
151734	<i>yadL</i>	A	C	Val166Val	-	-	-	-	0.26	-	-	-	-	-	-	-	-	-	-	-	-	-
2725790	<i>from_kgtP_</i> <i>to_yfiS</i>	T	G	-	-	-	-	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-
2725794	<i>from_kgtP_</i> <i>to_yfiS</i>	G	T	-	-	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-
3136631	<i>from_pitB_</i> <i>to_gss</i>	T	G	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-
4641647	<i>after_yjtD</i>	T	TTTTTCAGC	-	-	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	-	1.00	-	-	-
4104875	<i>cpxA</i>	T	G	Gln34Pro	-	-	-	-	-	0.97	-	-	-	-	-	-	-	-	-	-	-	-
1123119	<i>yceB</i>	G	C	Ala52Gly	-	-	-	-	-	0.37	-	-	-	-	-	-	-	-	-	-	-	-
497569	<i>adk</i>	T	G	Val132Gly	-	-	-	-	0.29	0.20	-	-	0.19	-	-	-	-	-	-	-	-	0.23
4082687	<i>fdoH</i>	C	G	Asp25His	-	-	-	-	-	0.17	-	0.21	-	-	-	-	-	-	-	-	-	-
3222500	<i>from_ebgR_</i> <i>to_ebgA</i>	T	G	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-	-
4104924	<i>cpxA</i>	CCAGCGT	C	Thr16 Leu17del	-	-	-	-	-	-	-	-	0.98	-	-	-	-	-	-	-	-	-
3817289	<i>yicC</i>	T	C	Leu205Pro	-	-	-	-	-	-	-	-	0.61	-	-	-	-	-	-	-	-	-
289278	<i>from_xynR_</i> <i>to_argF</i>	G	C	-	-	-	0.37	-	-	-	0.29	0.36	-	-	0.41	0.37	-	-	-	0.49	0.29	
2867272	<i>rpoS</i>	T	A	Met94Leu	-	-	-	-	-	-	-	-	0.25	-	-	-	-	-	-	-	-	-
1286793	<i>yehS</i>	A	G	Lys29Glu	-	-	-	-	-	-	-	-	0.24	-	0.21	0.41	0.28	-	-	-	-	-
2725807	<i>from_kgtP_</i> <i>to_yfiS</i>	G	T	-	-	-	-	-	-	-	-	-	0.21	-	-	-	-	-	-	-	-	-
1188951	<i>phoQ</i>	T	G	Thr276Pro	-	-	-	-	-	-	-	-	0.21	-	-	-	-	-	-	-	-	-
2948173	<i>amiC</i>	A	G	Phe280Leu	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-	-
306815	<i>ecpC</i>	C	T	Glu840Lys	-	-	-	-	-	-	-	-	0.18	-	-	-	-	-	-	-	-	-
3340800	<i>yrbG</i>	T	G	Phe176Val	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-	-	-	-	-
2153805	<i>from_ibsB_</i> <i>to_mdtA</i>	G	A	-	-	-	-	-	-	-	-	-	0.11	-	-	0.11	-	-	-	-	-	-
2312651	<i>ompC</i>	AT	A	Asp33fs	-	-	-	-	-	-	-	-	-	0.97	-	1.00	-	-	-	-	-	-
3535126	<i>envZ</i>	G	T	Pro248Leu	-	-	-	-	-	-	-	-	-	0.97	-	1.00	-	-	-	-	-	-
3713532	<i>tag</i>	G	T	Lys147Asn	-	-	-	-	-	-	-	-	-	0.96	1.00	0.99	-	-	-	-	-	-
290630	<i>ykgS</i>	A	C	Thr41Pro	-	-	-	-	-	-	-	-	-	0.26	-	-	-	-	-	-	-	-
290632	<i>ykgS</i>	C	T	Thr41Thr	-	-	-	-	-	-	-	-	-	0.26	-	-	-	-	-	-	-	-
290627	<i>ykgS</i>	G	A	Val40Ile	-	-	-	-	-	-	-	-	-	0.26	-	-	-	-	-	-	-	-
2570475	<i>eutE</i>	A	C	Leu426Arg	-	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-
4048768	<i>polA</i>	A	C	Lys601Asn	-	-	-	-	-	-	-	-	-	0.14	-	-	-	-	-	-	-	-
126360	<i>aceF</i>	T	C	Thr222Thr	-	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-
4360944	<i>cadC</i>	G	C	Pro331Ala	-	-	-	-	-	-	-	-	-	-	-	0.33	-	-	-	-	-	-
2521008	<i>from_valU_</i> <i>to_valX</i>	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.92	-	0.67	0.90	
2521007	<i>from_valU_</i> <i>to_valX</i>	A	C	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19	0.86	-	0.69	0.82	
4097445	<i>rhaB</i>	T	A	Thr2Ser	-	-	-	-	-	-	-	-	-	-	-	-	-	0.75	0.67	-	0.85	
986471	<i>ompF</i>	TC	T	Asp171fs	-	-	-	-	-	-	-	-	-	-	-	-	-	0.67	-	-	-	
4412779	<i>yjFM</i>	T	G	Val131Val	-	0.32	-	-	-	-	-	-	-	-	-	-	-	0.32	-	-	-	-
2521010	<i>from_valU_</i> <i>to_valX</i>	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	0.86	-	
3214385	<i>rpoD</i>	G	C	Ala447Pro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.59	-	
2521013	<i>from_valU_</i> <i>to_valX</i>	T	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	

2521050	<i>from_valU_ to_valX</i>	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.45	0.44
497572	<i>adk</i>	A	T	Tyr133Phe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.27	-
1080910	<i>from_putP_ to_efeO</i>	A	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.26	-
2521011	<i>from_valU_ to_valX</i>	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
2521012	<i>from_valU_ to_valX</i>	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
2521007	<i>from_valU_ to_valX</i>	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.09
2521009	<i>from_valU_ to_valX</i>	T	TAA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14	-
1397674	<i>uspE</i>	A	T	Ter317Lys	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14	-
2817745	<i>from_yqaB_ to_argQ</i>	T	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-
2818433	<i>from_argY_ to_argV</i>	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-
2818442	<i>from_argY_ to_argV</i>	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-

Table S2 Identified transposition and deletions in evolved strains

Position	Transposition and deletion	Referred gene	WT_R1_FT	WT_R1_AT	WT_R2_FT	WT_R2_AT	WT_R3_FT	WT_R3_AT	WT_R4_FT	WT_R4_AT	WT_R5_FT	WT_R5_AT	WT_R6_FT	WT_R6_AT	WT_R7_FT	WT_R7_AT	WT_R8_FT	WT_R8_AT	WT_R9_FT	WT_R9_AT	$\Delta ampC_{A2}$ _FT
2312791	IS1 (+) +9 bp	<i>from_ompC_ to_micF</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
986977	IS1 (+) +9 bp	<i>ompF</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1979427	IS1 (+) +9 bp	<i>from_insA5_ to_uspC</i>	-	-	-	-	0.84	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3623442	IS1 (+) +9 bp	<i>yhhH</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3803162	IS1 (-) +8 bp	<i>waaB</i>	-	-	-	-	0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4531699	IS1 (+) +9 bp	<i>from_sgcX_ to_yjhP</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4360945	IS1 (-) +8 bp	<i>cadC</i>	-	-	-	-	-	-	-	-	-	-	0.9	-	1	-	-	-	-	-	-
371758	IS30 (+) +2 bp	<i>mhpC</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-
1869100	IS1 (+) +8 bp	<i>yeaH</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-
986937	IS5 (-) +4 bp	<i>ompF</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-	-

1299499 to 1300698	Deletion	<i>insH21</i>	1	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
4092661 to 4104210	Deletion	<i>[frvA], rhaM, rhaD, rhaA, rhaB, rhaS, rhaR, rhaT, sodA, kdgT, yiiM, [cpxA]</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3998113 to 3998206	Deletion	<i>uvrD</i>	0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4184042 to 4184054	Deletion	<i>rpoB</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4104929 to 4104935	Deletion	<i>cpxA</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
2305470 to 2306485	Deletion	<i>ompC</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table S3 Single-nucleotide polymorphisms in evolved $\Delta ampC$

Position	Annotation	Reference base	Altered base	Altered amino acid	Frequency		
					$\Delta ampC$ 1 FT	$\Delta ampC$ 2 FT	$\Delta ampC$ 3 FT
88669	<i>ftsI</i>	C	T	Ala257Val	1.00	-	1.00
478037	<i>acrB</i>	G	A	Ser608Leu	1.00	-	1.00
4094389	<i>cpxA</i>	A	G	Met172Thr	1.00	-	-
3433607	<i>rpoA</i>	T	C	Glu261Gly	0.99	-	-
1188957	<i>mnmA</i>	T	C	Lys91Lys	0.99	-	-
4387462	<i>mutL</i>	T	A	Ser78Arg	0.98	-	-
3528528	<i>envZ</i>	G	A	Arg234Cys	0.97	-	1.00
2814783	<i>alaS</i>	G	C	Gly196Gly	0.82	-	-
2306322	<i>from ompC to micF</i>	CA	C	-	0.82	-	-
4089625	<i>rhaT</i>	C	T	Ala277Pro	0.81	-	-
4180206	<i>yjaZ</i>	G	A	Ala165Thr	0.81	-	-
1251288	<i>yegV</i>	A	G	Ser41Pro	0.78	-	-
3857063	<i>from yidL to yidP</i>	G	A	-	0.76	-	-
833004	<i>from ybiC to ybiJ</i>	A	G	-	0.52	-	-
3485056	<i>from yhfG to ppiA</i>	C	G	-	0.49	0.35	0.38
1588264	<i>from hipB to lsrK</i>	C	T	-	0.47	-	-
89121	<i>ftsI</i>	T	C	Ser408Pro	0.43	-	-
579935	<i>from appY to ompT</i>	G	GT	-	0.42	-	-
3227077	<i>from fadH to higA</i>	G	C	-	0.41	0.44	0.53
3485060	<i>from yhfG to ppiA</i>	C	G	-	0.40	-	-

284989	<i>from yagI to argF</i>	G	C	-	0.39	0.34	0.30
962370	<i>msbA</i>	G	A	Met98Ile	0.34	-	-
1282249	<i>from ori to rttR</i>	A	G	-	0.29	0.25	0.27
1014424	<i>from matP to ompA</i>	C	CA	-	0.24	-	-
1481977	<i>ydcF</i>	G	A	Thr162Thr	0.23	-	-
93562	<i>mraY</i>	G	T	Lys358Asn	0.21	-	-
1304694	<i>from kch to yciI</i>	G	A	-	0.20	-	-
3977523	<i>aslB</i>	G	A	Met402Ile	0.19	-	-
2512691	<i>from yfeD to gltX</i>	A	C	-	0.19	-	-
2811133	<i>from yqaB to argQ</i>	A	T	-	0.17	0.19	0.14
353289	<i>from codA to cynR</i>	T	C	-	0.17	-	-
198945	<i>fabZ</i>	A	C	Thr120Pro	0.16	-	-
776694	<i>from lysY to lysZ</i>	T	A	-	0.16	-	-
353286	<i>from codA to cynR</i>	T	A	-	0.16	-	-
2514608	<i>from valY to lysV</i>	CT	C	-	0.16	-	-
353317	<i>from codA to cynR</i>	C	T	-	0.14	-	-
1941441	<i>ruvC</i>	C	T	Ala65Thr	0.13	-	-
731810	<i>from ybfC to ybfD</i>	A	T	-	0.13	0.12	0.11
1613585	<i>marR</i>	C	T	Ala70Val	0.13	-	-
3781445	<i>envC</i>	G	A	Trp416*	0.13	-	-
3595746	<i>ftsE</i>	G	A	Ala121Val	0.13	-	-
422846	<i>yajC</i>	A	G	Tyr35Cys	0.12	-	-
731939	<i>from ybfC to ybfD</i>	A	T	-	0.12	0.10	-
353337	<i>from codA to cynR</i>	C	T	-	0.12	-	-
2147286	<i>from ibsB to mdtA</i>	G	A	-	0.12	0.11	-
3188443	<i>from ibsD to sibE</i>	A	G	-	0.12	-	-
2306035	<i>ompC</i>	GT	G	Thr65fs	0.11	-	-
353338	<i>from codA to cynR</i>	C	A	-	0.11	-	-
477843	<i>acrB</i>	C	T	Glu673Lys	0.10	-	-
477976	<i>acrB</i>	G	T	Phe628Leu	-	1.00	-
479457	<i>acrB</i>	T	G	Ser135Arg	-	1.00	-
2306481	<i>from ompC to micF</i>	TTAA	T	-	-	1.00	-
3528023	<i>envZ</i>	G	T	Thr402Lys	-	1.00	-
89506	<i>ftsI</i>	A	T	Gln536Leu	-	0.99	-
2306477	<i>from ompC to micF</i>	A	T	-	-	0.82	-
2222655	<i>from yohP to dusC</i>	C	G	-	-	0.60	0.53
2222656	<i>from yohP to dusC</i>	A	G	-	-	0.58	0.52
910683	<i>from ybjE to aqpZ</i>	G	C	-	-	0.56	0.64
910682	<i>from ybjE to aqpZ</i>	T	C	-	-	0.52	-
2222662	<i>from yohP to dusC</i>	C	G	-	-	0.51	0.42
910676	<i>from ybjE to aqpZ</i>	G	C	-	-	0.42	0.60
2222665	<i>from yohP to dusC</i>	C	G	-	-	0.42	0.36
481184	<i>from acrA to acrR</i>	AT	A	-	-	0.40	-
481176	<i>from acrA to acrR</i>	A	AGGGGG	-	-	0.32	-
1205075	<i>from tfaE to pinE</i>	C	G	-	-	0.32	-
4631464	<i>after yjiD</i>	T	TTTTTCAGCTTTTCA	-	-	0.31	-
2730284	<i>from bamD to raiA</i>	T	G	-	-	0.22	-
2306477	<i>from ompC to micF</i>	A	C	-	-	0.18	-
122568	<i>aceF</i>	T	C	Asp129Asp	-	0.16	-
122562	<i>aceF</i>	T	G	Val127Val	-	0.15	-

1460401	<i>from ynbG to insD1</i>	G	T	-	-	0.15	-
1460395	<i>from ynbG to insD1</i>	G	A	-	-	0.14	-
731817	<i>from ybfC to ybfD</i>	C	A	-	-	0.14	-
122880	<i>aceF</i>	C	A	Ala233Ala	-	0.14	-
3187978	<i>from yqiK to sibD</i>	A	G	-	-	0.13	-
3187962	<i>from yqiK to sibD</i>	A	C	-	-	0.13	-
1546447	<i>from fdnI to yddM</i>	T	C	-	-	0.12	-
2147307	<i>from ibsB to mdtA</i>	A	C	-	-	0.12	-
2147312	<i>from ibsB to mdtA</i>	A	T	-	-	0.12	-
3187990	<i>from yqiK to sibD</i>	G	C	-	-	0.11	-
2147254	<i>sibB;from ibsB to mdtA</i>	G	A	-	-	0.11	-
2147247	<i>sibB;from ibsB to mdtA</i>	T	C	-	-	0.11	-
3433613	<i>rpoA</i>	T	G	Asp259Ala	-	-	0.99
2305858	<i>ompC</i>	C	T	Trp124*	-	-	0.96
2711680	<i>yfiF</i>	G	C	Arg70Gly	-	-	0.42
1205058	<i>from tfaE to pinE</i>	G	GTTTGGCT	-	-	-	0.32
1205055	<i>from tfaE to pinE</i>	A	ATT	-	-	-	0.31
1282287	<i>from OrI to rttR</i>	T	A	-	-	-	0.31
2170700	<i>from gatY to fbaB</i>	T	G	-	-	-	0.22
1892012	<i>sdaA</i>	A	C	Asn275Thr	-	-	0.20
1460515	<i>from ynbG to insD1</i>	G	C	-	-	-	0.19
3413745	<i>yhdX</i>	A	T	Ile85Phe	-	-	0.16
2811104	<i>from yqaB to argQ</i>	T	C	-	-	-	0.16
2811097	<i>from yqaB to argQ</i>	A	T	-	-	-	0.15
2811130	<i>from yqaB to argQ</i>	G	A	-	-	-	0.14
2387247	<i>nuoL</i>	G	T	Leu427Ile	-	-	0.11

Table S4 Mutations in *ampC* promoter in evolved WT at different time points during the amoxicillin evolution

Evolved days	R1	R2	R3	R4	R5	R6	R7	R8	R9
1	-	-	-	-	-	-	-	-	-
2	-11 G>A	-11 G>A	-11 G>A	-11 G>A	-11 G>A	-11 G>A	-11 G>A	-	-11 G>A
3	-11 G>A	-11 G>A	-11 G>A	-11 G>A	+54 C>T, +20 G>C, +11 A>C, +7 T>G, +4 C>G, -2 C>G, -11 G>A, -15 C>G, -17A>C	-11 G>A	-11 G>A	-	-11 G>A
6	-11 G>A	-11 G>A	-11 G>A	-11 G>A, +31 del G	-25_-26 ins A, +7 del T	-11 G>A	-11 G>A	-32 A>T	-11 G>A
9	-11 G>A, +35 G>T	-11 G>A, +32 del C	-11 G>A, +37 C>T	-11 G>A, +31 del G	-25_-26 ins A	+32 to +38 del, -32 A>T	-11 G>A, +59 A>G, +56G>T, +54 C>T, +53 T>G	-32 A>T	-32 A>T
12	-11 G>A, +33 C>A +35 G>T	-11 G>A, +32 del C	-11 G>A	-11 G>A, +31 del G	-25_-26 ins A	+32 to +38 del, -32 A>T, -7 G>T, +9A>T, +47_+46 ins TC	-11 G>A	-32 A>T	-32 A>T
16	-11 G>A, +33 C>A	-11 G>A, +32 del C	-11 G>A, +7 del T	-11 G>A, +7 del T, +31 del G	-25_-26 ins A, +7 del T	+32 to +38 del, -32 A>T,	-11 G>A, +23 C>A, +41A>G, +55T>A	-32 A>T, +29 to +32 del	-32 A>T

22	-11 G>A, +33 C>A	-11 G>A, +32 del C	-11 G>A	-11 G>A, +31 del G	-25_-26 ins A	+47_+46 ins TC +32 to +38 del, -32 A>T, +47_+46 ins TC	-11 G>A, +23 C>A	-32 A>T	-32 A>T
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Table S5 Primers and probes used in this study

Primers/Probes	Sequence
ampC_F	ACTGGGGTTGTTTCCGGGTG
ampC_R	TGGTTGAGTTTGAGTGGCTG
gadph_F	GCTGAAGGCGAAATGAAAGG
gadph_R	GTACCAGGATACCAGTTTCACG
F-AmpC Prom	CGCCGGTGATGATCCTGCTGGT
R-AmpC prom	ATGTTTTGCTGACCGAACCTAA
ampC_probe	/56-FAM/ATTAAGTGCCTGCAACACCACGC/36-TAMSp/
gadph_probe	/56-FAM/CTGGACGTGGTTGAAGGTATGCAGT/36-TAMSp/