

Supplementary Information

Proteomic Investigation of Tolerant *Escherichia coli* Populations from Cyclic Antibiotic Treatment

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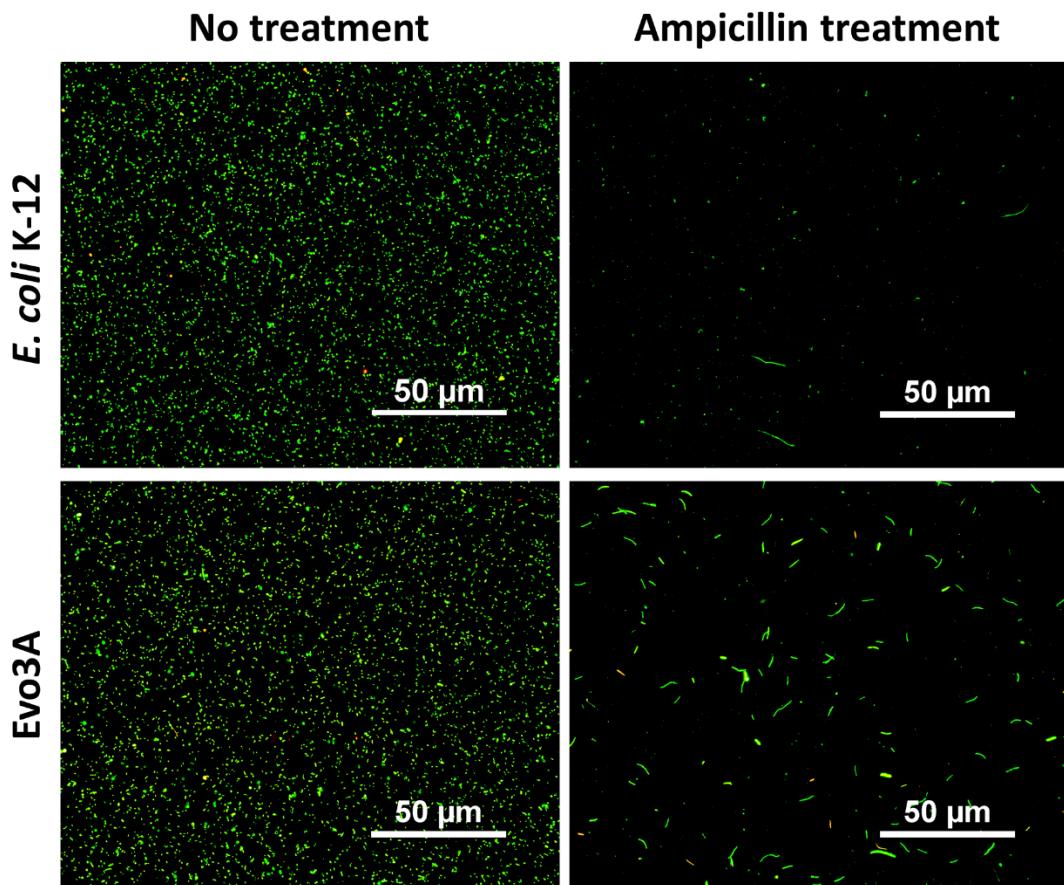


Fig. S1. *Evo3A* filamented homogenously upon ampicillin treatment indicating that an SOS response is required for survival. *E. coli* K-12 and *Evo3A* were stained with a LIVE/DEAD kit and visualized by epifluorescence microscopy before and after 3 h of ampicillin treatment (12.5× MIC).

e14 Integration to *icd* gene

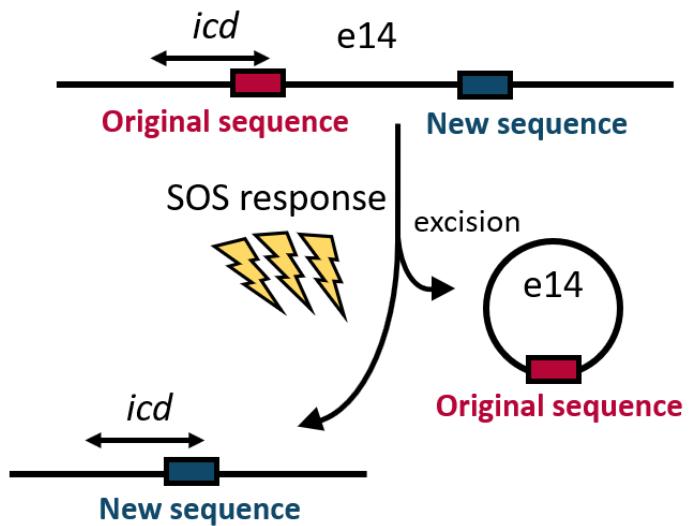


Fig. S2. Integration event of e14 prophage to the *icd* gene. The picture indicates the region of *E. coli* K-12 chromosome containing *icd* and e14. The red and blue boxes indicate the 216-bp homologies and the arrows indicate the positions involved in the crossover leading to excision after SOS response is triggered. This integration event was described by Hill et al¹.

Supplementary Tables

Table S1. Strains used to verify the important proteins for persistence based on the proteomics result.

Strain	CGSC #	Relevant genotype/comments	ref.
<i>E. coli</i> K-12			
BW25113 (WT)	7636	K-12 derivative: $\Delta araBAD$, $\Delta rhaBAD$	2
JW0599-1 ($\Delta ahpF$)	8714	BW25113 derivative: $\Delta ahpF745::kan$	3
JW2279-3 ($\Delta nuoF$)	9837	BW25113 derivative: $\Delta nuoF766::kan$	3
JW2418-2 ($\Delta cysP$)	9926	BW25113 derivative: $\Delta cysP774::kan$	3

Table S2. Single point mutations on the *icd* gene related to the integration of e14 prophage.

Strain	Sequence ID	Genomic position	Mutation	Amino acid substitution	Gene	Annotation
Evo3C	NC_000913.3	1,196,220	C>T	His366His (CAC-> CAU)	<i>icd</i>	Isocitrate dehydrogenase
		1,196,232	C>T	Thr370Thr (ACC-> ACU)		
		1,196,245	T>C	Leu375Leu (UUA-> CUA)		
		1,196,247	A>G	Leu375Leu (UUA-> UUG)		
		1,196,277	C>T	Asn385Asn (AAC-> AAU)		
		1,196,280	G>C	Ala386Ala (GCG-> GCC)		
		1,196,283	A>G	Lys387Lys (AAA-> AAG)		
		1,196,292	C>T	Thr390Thr (ACC-> ACU)		
		1,196,304	G>A	Glu394Glu (GAG-> GAA)		
		1,196,316	T>A	Asp398Glu (GAU-> GAA)		

Table S3. Proteins exclusively identified on the three evolved populations

UniProt ID	Gene	Protein name
P0A6P5	<i>der</i>	GTPase Der
P11349	<i>narH</i>	Respiratory nitrate reductase 1 beta chain
P0ADS6	<i>yggE</i>	Uncharacterized protein YggE
P00582	<i>polA</i>	DNA polymerase I
Q06065	<i>atoC</i>	Regulatory protein AtoC
P0AF03	<i>mog</i>	Molybdopterin adenylyltransferase
P04951	<i>kdsB</i>	3-deoxy-manno-octulosonate cytidylyltransferase
P0AF12	<i>mtnN</i>	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
P0ABU9	<i>tolQ</i>	Tol-Pal system protein TolQ
P0ACC3	<i>erpA</i>	Iron-sulfur cluster insertion protein ErpA
P0A7L8	<i>rpmA</i>	50S ribosomal protein L27
P06989	<i>hisI</i>	Histidine biosynthesis bifunctional protein HisIE
P61714	<i>ribE</i>	6,7-dimethyl-8-ribityllumazine synthase
P37759	<i>rfbB</i>	dTDP-glucose 4,6-dehydratase 1
P0ADF8	<i>ilvN</i>	Acetolactate synthase isozyme 1 small subunit
P0A9M2	<i>hpt</i>	Hypoxanthine phosphoribosyltransferase
P28249	<i>asmA</i>	Protein AsmA
P06987	<i>hisB</i>	Histidine biosynthesis bifunctional protein HisB
P0A7U3	<i>rpsS</i>	30S ribosomal protein S19
P20083	<i>parE</i>	DNA topoisomerase 4 subunit B
P0A7I4	<i>prfC</i>	Peptide chain release factor RF3
P0AFJ7	<i>pitA</i>	Low-affinity inorganic phosphate transporter 1

Table S4. Differentially expressed proteins of Evo3A compared to ancestral population.

UniProt ID	p-value	Folds	Gene	Protein name
P42632	0.0153	0.37	<i>tdcE</i>	PFL-like enzyme TdcE
P02931	0.0014	0.52	<i>ompF</i>	Outer membrane protein F
P37903	0.0080	0.54	<i>uspF</i>	Universal stress protein F
P08194	0.0057	0.55	<i>glpT</i>	Glycerol-3-phosphate transporter
P0A9C0	0.0237	0.56	<i>glpA</i>	Anaerobic glycerol-3-phosphate dehydrogenase subunit A
P00805	0.0006	0.56	<i>ansB</i>	L-asparaginase 2
P0C8J6	0.0011	0.59	<i>gatY</i>	D-tagatose-1,6-bisphosphate aldolase subunit GatY
P76177	0.0455	0.59	<i>ydgH</i>	Protein YdgH
P0ADZ7	0.0122	0.64	<i>yajC</i>	Sec translocon accessory complex subunit YajC
P31658	0.0093	1.50	<i>hchA</i>	Protein/nucleic acid deglycase 1
P23827	0.0126	1.52	<i>eco</i>	Ecotin
P0A867	0.0206	1.52	<i>talA</i>	Transaldolase A
P25894	0.0170	1.54	<i>loiP</i>	Metalloprotease LoiP
P0A991	0.0079	1.59	<i>fbaB</i>	Fructose-bisphosphate aldolase class 1
P0A805	0.0141	1.59	<i>frr</i>	Ribosome-recycling factor
P68206	0.0095	1.70	<i>yjbJ</i>	UPF0337 protein YjbJ
P16700	0.0375	1.71	<i>cysP</i>	Thiosulfate-binding protein
P09152	0.0237	1.77	<i>narG</i>	Respiratory nitrate reductase 1 alpha chain
P0AD59	0.0180	1.84	<i>ivy</i>	Inhibitor of vertebrate lysozyme
P18843	0.0142	1.88	<i>nadE</i>	NH(3)-dependent NAD(+) synthetase
P07003	0.0153	1.93	<i>poxB</i>	Pyruvate dehydrogenase
P31979	0.0402	2.11	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F
P35340	0.0279	2.20	<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F
P37665	0.0021	2.31	<i>yiaD</i>	Probable lipoprotein YiaD
P65292	0.0201	2.42	<i>ygdI</i>	Uncharacterized lipoprotein YgdI

P36659	0.0492	2.80	<i>cbpA</i>	Curved DNA-binding protein
P31677	0.0130	2.84	<i>otsA</i>	Trehalose-6-phosphate synthase
P69908	0.0198	2.84	<i>gadA</i>	Glutamate decarboxylase alpha
P77581	0.0173	3.29	<i>astC</i>	Succinylornithine transaminase
P77454	0.0076	4.39	<i>glsA1</i>	Glutaminase 1

Table S5. Differentially expressed proteins of Evo3C compared to ancestral population.

UniProt ID	p-value	Folds	Gene	Protein name
P09551	0.0012	0.19	<i>argT</i>	Lysine/arginine/ornithine-binding periplasmic protein
P76536	0.0020	0.29	<i>yfeX</i>	Dye-decolorizing peroxidase YfeX
P09169	0.0217	0.43	<i>ompT</i>	Protease 7
P75691	0.0279	0.52	<i>yahK</i>	Aldehyde reductase YahK
P02931	0.0029	0.53	<i>ompF</i>	Outer membrane protein F
P31663	0.0242	0.56	<i>panC</i>	Pantothenate synthetase
P27550	0.0495	0.56	<i>acs</i>	Acetyl-coenzyme A synthetase
P17169	0.0168	0.57	<i>glmS</i>	Glutamine--fructose-6-phosphate aminotransferase
P0AA53	0.0418	0.62	<i>qmcA</i>	Protein QmcA
P0A9G6	0.0337	0.63	<i>aceA</i>	Isocitrate lyase
P0ADZ7	0.0081	0.65	<i>yajC</i>	Sec translocon accessory complex subunit YajC
P0AFH8	0.0319	0.65	<i>osmY</i>	Osmotically-inducible protein Y
P09373	0.0063	1.51	<i>pflB</i>	Formate acetyltransferase 1
P0AFC3	0.0311	1.53	<i>nuoA</i>	NADH-quinone oxidoreductase subunit A
P0A9Q7	0.0107	1.53	<i>adhE</i>	Aldehyde-alcohol dehydrogenase
P0AC33	0.0049	1.58	<i>fumA</i>	Fumarate hydratase class I
P42632	0.0329	1.60	<i>tdcE</i>	PFL-like enzyme TdcE
P0C8J6	0.0027	1.61	<i>gatY</i>	D-tagatose-1,6-bisphosphate aldolase subunit GatY
P0A7R9	0.0451	1.80	<i>rpsK</i>	30S ribosomal protein S11
P36938	0.0177	1.81	<i>pgm</i>	Phosphoglucomutase
P23827	0.0362	1.83	<i>eco</i>	Ecotin
P16700	0.0123	1.94	<i>cysP</i>	Thiosulfate-binding protein
P33602	0.0496	2.00	<i>nuoG</i>	NADH-quinone oxidoreductase subunit G
P37665	0.0113	2.05	<i>yiaD</i>	Probable lipoprotein YiaD
P35340	0.0095	2.15	<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F

P0AD49	0.0380	2.18	<i>raiA</i>	Ribosome-associated inhibitor A
P09546	0.0020	2.22	<i>putA</i>	Bifunctional protein PutA
P69797	0.0044	2.25	<i>manX</i>	PTS system mannose-specific EIIAB component
P0A996	0.0070	2.29	<i>glpC</i>	Anaerobic glycerol-3-phosphate dehydrogenase subunit C
P37329	0.0465	2.56	<i>modA</i>	Molybdate-binding protein ModA
P31979	0.0065	2.59	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F
P07012	0.0423	2.60	<i>prfB</i>	Peptide chain release factor RF2
P33363	0.0400	2.85	<i>bgIX</i>	Periplasmic beta-glucosidase
P0AB89	0.0309	2.97	<i>purB</i>	Adenylosuccinate lyase
P68066	0.0047	2.97	<i>grcA</i>	Autonomous glycyl radical cofactor

Table S6. Differentially expressed proteins of Evo3P compared to ancestral population.

UniProt ID	p-value	Folds	Gene	Protein name
P39180	0.0002	0.11	<i>flu</i>	Antigen 43
P23847	0.0004	0.15	<i>dppA</i>	Periplasmic dipeptide transport protein
P42632	0.0175	0.21	<i>tdcE</i>	PFL-like enzyme TdcE
P75691	0.0028	0.21	<i>yahK</i>	Aldehyde reductase YahK
P25516	0.0028	0.22	<i>acnA</i>	Aconitate hydratase A
P07003	0.0155	0.22	<i>poxB</i>	Pyruvate dehydrogenase
P0ADX7	0.0407	0.24	<i>yhhA</i>	Uncharacterized protein YhhA
P0A996	0.0457	0.25	<i>glpC</i>	Anaerobic glycerol-3-phosphate dehydrogenase subunit C
P0A991	0.0011	0.27	<i>fbaB</i>	Fructose-bisphosphate aldolase class 1
P13035	0.0070	0.27	<i>glpD</i>	Aerobic glycerol-3-phosphate dehydrogenase
P09551	0.0095	0.32	<i>argT</i>	Lysine/arginine/ornithine-binding periplasmic protein
P42620	0.0365	0.32	<i>yqjG</i>	Glutathionyl-hydroquinone reductase YqjG
P25553	0.0111	0.42	<i>aldA</i>	Lactaldehyde dehydrogenase
P0A867	0.0131	0.43	<i>talA</i>	Transaldolase A
P0ADU5	0.0361	0.45	<i>ygiW</i>	Protein YgiW
P76558	0.0198	0.46	<i>maeB</i>	NADP-dependent malic enzyme
P27550	0.0141	0.46	<i>acs</i>	Acetyl-coenzyme A synthetase
P37194	0.0190	0.51	<i>slp</i>	Outer membrane protein Slp
P0A9G6	0.0273	0.52	<i>aceA</i>	Isocitrate lyase
P08997	0.0064	0.54	<i>aceB</i>	Malate synthase A
P0A7U7	0.0383	0.54	<i>rpsT</i>	30S ribosomal protein S20
P23843	0.0120	0.54	<i>oppA</i>	Periplasmic oligopeptide-binding protein
P31658	0.0039	0.55	<i>hchA</i>	Protein/nucleic acid deglycase 1
P0AET2	0.0042	0.55	<i>hdeB</i>	Acid stress chaperone HdeB
P37636	0.0314	0.59	<i>mdtE</i>	Multidrug resistance protein MdtE

P0ACF4	0.0140	0.60	<i>hupB</i>	DNA-binding protein HU-beta
P0A836	0.0457	0.61	<i>sucC</i>	Succinate--CoA ligase
P0A6M8	0.0357	0.61	<i>fusA</i>	Elongation factor G
P19926	0.0189	0.62	<i>agp</i>	Glucose-1-phosphatase
P0A6F3	0.0168	0.63	<i>glpK</i>	Glycerol kinase
P09169	0.0262	1.53	<i>ompT</i>	Protease 7
P00961	0.0159	1.69	<i>glyS</i>	Glycine-tRNA ligase beta subunit
P68066	0.0230	1.69	<i>grcA</i>	Autonomous glycyl radical cofactor
P0A805	0.0176	1.91	<i>frr</i>	Ribosome-recycling factor
P0AD49	0.0368	2.10	<i>raiA</i>	Ribosome-associated inhibitor A
P0ADG7	0.0067	2.36	<i>guaB</i>	Inosine-5'-monophosphate dehydrogenase
P07012	0.0254	2.49	<i>prfB</i>	Peptide chain release factor RF2
P00962	0.0001	3.26	<i>glnS</i>	Glutamine-tRNA ligase
P0AEG6	0.0212	3.33	<i>dsbC</i>	Thiol:disulfide interchange protein DsbC

Table S7. Proteins with similar expression profile across all evolved populations.

Gene	Protein name	Fold changes			p-values		
		Evo3A	Evo3C	Evo3P	Evo3A	Evo3C	Evo3P
<i>grcA</i>	Autonomous glycyl radical cofactor	1.21	2.97	1.69	0.102	0.005	0.023
<i>raiA</i>	Ribosome-associated inhibitor A	2.05	2.18	2.10	0.056	0.038	0.037
<i>frr</i>	Ribosome-recycling factor	1.59	1.38	1.91	0.014	0.008	0.018
<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F	2.20	2.15	1.76	0.028	0.009	0.171
<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	2.11	2.59	1.39	0.040	0.006	0.401
<i>cysP</i>	Thiosulfate-binding protein	1.71	1.94	1.89	0.037	0.012	0.213

References

- (1) Hill, C. W.; Gray, J. A.; Brody, H. Use of the isocitrate dehydrogenase structural gene for attachment of e14 in *Escherichia coli* K-12. *J. Bacteriol.* **1989**, *171*, 4083-4084.
- (2) Datsenko, K. A.; Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 6640-6645.
- (3) Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006**, *2*, 2006.0008.