Supplementary Information

Cryptic Prophages Help Bacteria Cope with Adverse Environments

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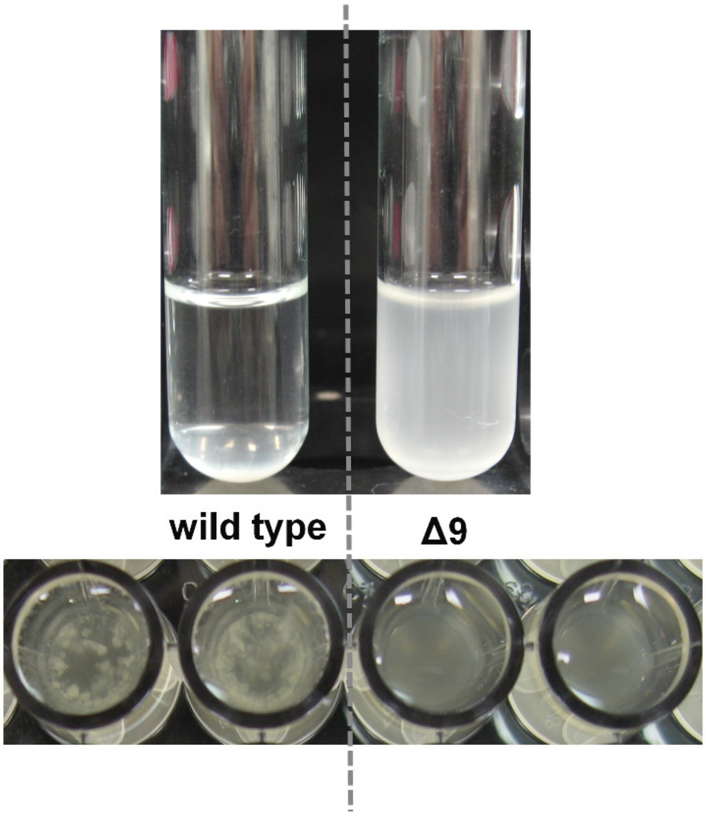

Supplementary Figure S1. Prophage genes increase cell aggregation. Aggregation for the multiple prophage-deletion strain (Δ9) and the wild-type strain. Bacteria were cultured at 30°C for 48 h in M9C medium in glass tubes (top) and in LB medium in 96-well polystyrene plates (bottom).

Supplementary Table S1. List of chemicals that affect the cellular respiration of Δ9 vs. wild-type BW25113 using Biolog PM Plates 9 and 11-13. Area difference is the average area under the growth curve for Δ9 vs. the wild-type strain (*n* = 3); i.e., negative values indicate the wild-type strain respires more robustly than Δ9.

PM Plate	Chemical	Area Difference	Mode of action
Fluroquinolones			
PM11	lomefloxacin	-21444	DNA unwinding, gyrase (GN), fluoroquinolone
PM11	ofloxacin	-35026	DNA unwinding, gyrase (GN), fluoroquinolone
PM13	oxolinic acid	-35915	DNA unwinding, gyrase (GN), quinolone
PM12	novobiocin	-22377	DNA unwinding, gyrase (GN), quinolone
PM11	nalidixic acid	-10711	DNA unwinding, gyrase (GN), quinolone
PM11	enoxacin	-4881	DNA unwinding, gyrase (GN), fluoroquinolone
β-lactams			
PM13	moxalactam	-27479	wall, lactam
PM13	azlocillin	-19146	wall, lactam
PM13	cefuroxime	-25228	wall, cephalosporin second generation
PM11	cephalothin	-24343	wall, cephalosporin first generation
PM11	nafcillin	-10824	wall, lactam
PM11	ceftriaxone	-22421	wall, cephalosporin third generation
PM13	ampicillin	-5427	wall, lactam
PM11	cloxacillin	-4505	wall, lactam
PM11	amoxicillin	-10776	wall, lactam
Ribosomal inhibitors			
PM11	kanamycin	-21349	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM11	gentamicin	-9166	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM11	amikacin	-10891	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM12	paromomycin	-11604	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM11	capreomycin	-47950	protein synthesis
PM11	erythromycin	-13645	protein synthesis, 50S ribosomal subunit, macrolide
PM11	chloramphenicol	-15743	protein synthesis
PM13	tylosin	-9589	protein synthesis, 50S ribosomal subunit, macrolide
Osmolytes			
PM09	3% NaCl	-5507	osmotic sensitivity
PM09	4% NaCl	-12946	osmotic sensitivity
PM09	5% NaCl	-35902	osmotic sensitivity
PM09	5.5% NaCl	-31073	osmotic sensitivity
PM09	6% NaCl	-13963	osmotic sensitivity
PM09	6.5% NaCl	-21400	osmotic sensitivity
PM09	6% NaCl +X	-24663	osmotic sensitivity, NaCl control and with "X"
PM09	6% NaCl +Y	-27021	osmotic sensitivity, NaCl control and with "Y"
PM09	5% potassium chloride	-14031	osmotic sensitivity
PM09	6% potassium chloride	-35572	osmotic sensitivity
PM09	2% sodium formate	-9195	osmotic sensitivity
PM09	2% sodium lactate	-14588	osmotic sensitivity
Others			
PM11	bleomycin	-24537	DNA damage, oxidative, ionizing radiation
PM12	vancomycin	-22730	Wall
PM13	thallium (I) acetate	-24856	toxic cation
PM13	manganese (II) chloride	-7781	toxic cation

PM12	6-mercapto-purine	12793	inhibits purine nucleotide synthesis and metabolism
PM12	benzethonium chloride	38475	toxic cation

Supplementary Table S2. List of cryptic prophage genes that are differentially expressed upon adding nalidixic acid (2 µg ml^{-1}) and azlocillin (10 µg ml^{-1}) for 90 min to *E. coli* K-12 (full data available using GEO accession number GSE23359).

Gene name	b-number*	Fold-change (Nalidixic acid)	Fold-change (Azlocillin)	Description
DLP12				
emrE	b0543	2.14	2.30	Multidrug resistance pump
ybcN	b0547	3.25	3.25	Unknown
ninE	b0548	2.64	2.83	Homolog of phage lambda *nin*
quuD	b0551	2.64	2.83	Unknown
nmpC	b0553	-3.03	-2.83	Outer membrane porin protein
essD	b0554	2.83	2.64	Holin
ybcV	b0558	3.25	3.48	Putative an envelope protein
ybcW	b0559	2.46	2.64	Unknown
ylcE	b0563	2.83	2.83	Unknown
e14				
intE	b1140	2.30	2.30	e14 integrase
croE	b1146	2.30	2.30	Cro-like repressor
ymfM	b1148	2.30	2.30	Putative cell division inhibitor
ycfE	b1157	2.64	2.64	Putative tail fiber protein
pinE	b1158	2.46	2.83	DNA invertase, site-specific recombination
rac				
intR	b1345	2.30	2.64	rac integrase
ydaQ	b1346	2.83	3.03	Putative rac excisionase
ralR	b1348	2.30	2.64	Restriction alleviation and modification enhancement
racC	b1351	2.30	2.64	RacC protein
kilR	b1352	2.30	2.83	Killing function zygotic induction; cell division arrest
ydaY	b1366	2.30	2.83	Unknown
Qin				
stfQ	b1547	2.46	2.83	Side-tail fiber protein
ydfO	b1549	2.46	2.64	Unknown
ynfN	b1551	2.30	2.83	Cold shock-induced protein
cspI	b1552	2.30	2.83	Cold shock-like protein
rzpQ	b1553	2.46	3.48	Unknown
arrQ	b1554	2.00	2.64	Putative lysozyme
ydfR	b1555	2.30	2.83	Unknown
essQ	b1556	2.30	2.64	Putative holin
cspB	b1557	2.14	2.64	Cold shock-like protein
ydfU	b1560	2.14	2.83	Unknown
rem	b1561	2.30	2.64	Unknown
ydfV	b1565	2.30	2.64	Unknown
dicC	b1569	2.30	2.83	Regulator of *dicB*
dicF	b1574	2.14	2.46	Antisense RNA, inhibits *ftsZ* translation
dicB	b1575	2.30	2.64	Inhibition of cell division
ydfD	b1576	2.30	2.83	Unknown
ydfE	b1577	2.14	2.64	Unknown
CP4-44				
flu	b2000	-3.48	-3.25	Antigen 43, phase-variable
yeeU	b2004	2.64	2.64	Antitoxin of toxin-antitoxin pair YeeV-YeeU
CPS-53				
yfdL	b2355	2.64	2.64	Tail fiber protein
yfdR	b2361	3.03	3.03	Unknown
yfdT	b2363	2.3	2.64	Unknown
CP4-57				
alpA	b2624	2.46	2.64	Putative excisionase
yfjJ	b2626	2.64	2.64	Unknown

b-numbers* were assigned sequentially along the *E. coli* genome by the Blattner Laboratory as part of the *E. coli* genome project.

Supplementary Table S3. Oligonucleotides used for prophage excision by qPCR, for verification of deletions by DNA sequencing, for qRT-PCR, and for one-step inactivation of five of the prophages.

Purpose	Primer sequence (5' to 3')
qPCR and deletion verification	
CP4-6	GCATCGCCCGGTTAGTTTTA
	CACCTGCACTGCCTGATGTC
DLP12	CAAAAGCCATTGACTCAGCAAGG
	ACGGATAAGACGGGCATAAATGA
e14	GTGCAAACATCGGTGACGAA
	TTCAGCAGCTTAGCGCCTTC
Rac	CTCCAGCATGGTATAGCTGTCTTTAC
	CAGATTTCTTATGCTGGGCGTTCCG
Qin	CGACAATACGCGCCACATAA
	AACGGCGAGTAAGTAGTACGCA
CP4-44	GAATTCACTGTCGGCGAAGG
	TTGAAGCAATCCAGGGCATC
CPS-53	CGTACTTACCCCGCACTCCA
	GGCAGTGGCCAAAAATTGAA
CPZ-55	AGCACATCCCCCGAACG
	TTGACGAAGTGATTGTCCGC
CP4-57	AAGCATGTAGTACCGAGGATGTAGG
	TATGTCTCCTCACCGTCTGGTCGG
qRT-PCR	
rrsG	TATTGCACAATGGGCGCAAG
	ACTTAACAAACCGCTGCGT
rpoS	AGAGTAACTTGCGTCTGGTGGTAAA
	ATAGTACGGGTTTGGTTCATAATCG
oxyR	TGAGGTGAAAGTCCTTAAAGAGATG
	GTCTGTGCTTCATGCAGATACATT
katE	GATCTTCTCGATCCAACCAAAC
	CACCAAGACGACTGATTTGTGT
kilR	CCACGTCAGTGTGTGACTCC
	CCGCCTTTACAGGTAAACCA
dicB	GGTTGTTTTGAAATTGGTGTCA
	CATTGTGCACATCCTTTTGG
Knock-out prophage region	
DLP12	ACGACCTTCTAAGTCGTGGGCCGCAGGTTCGAATCCTGCAGGGCGCGCCAGTGTAGGCTGGAGCTGCTTC
	TGAAGGGCAAAGCGGAAACGGATAAGACGGGCATAAATGAGGAAGAAACATATGAATATCCTCCTTAG
Qin	CATGAAACACTAACGCTGCGCCGAGCGAATATAACTGGAAATCCATAAGTGTAGGCTGGAGCTGCTTC
	ATTTGGTGAAAGCAGCAGTATCGGGATGGCTGGGCACTGCGCTTGAATCATATGAATATCCTCCTTAG
CP4-6	GCTCAGTTGGTAGAGCAGCGCATTCGTAATGCGAAGGTCGTAGGTTCGGTGTAGGCTGGAGCTGCTTC
	TTAAATATGGGGTAACTCTTTGATTTTAATGGTGCCGATAATAGGAGTCATATGAATATCCTCCTTAG
CP4-44	TCCAGATGACAAACATGATCTCATATCAGGGACTTGTTCGCACCTTCCGTGTAGGCTGGAGCTGCTTC
	AAAAAGCTGAAAGCTATGGGCGAAATGAAAAACGGCGAAGCGAAGTAACATATGAATATCCTCCTTTAG
CPZ-55	TTGCCGAACAATGTGGTCTTTAGTTTCATAAGTCGTTCCCTCAGGAAGGTGTAGGCTGGAGCTGCTTC
	TTTTTGGCGGATCGGTTGTGCCGGTGACGGTGAAATCACTCGCATTTCCATATGAATATCCTCCTTTAG

*Underlined region indicates nucleotides at the 5'-end of the prophage containing the right attachment site. Other nucleotides are for homology with pKD4 (source of the kanamycin resistance gene).