

Supplementary Table 1: Bacterial strains used in this study.

Nomenclature	Species/serotype/genotype	Source
042	Prototypical enteroaggregative <i>E. coli</i> strain (O44:H18)	M. D. Goldberg (BCU) [14]
1070/13	Enteroaggregative <i>E. coli</i> O104:H4 strain 1070/13	[1]
1070/13 $\Delta aggR$	EAEC 1070/13, $\Delta aggR::cat$	This study
1070/13 $\Delta ccdB$	EAEC 1070/13, $\Delta ccdB::cat$	This study
1070/13 pAA ⁻	Plasmid-free derivative of EAEC 1070/13	This study
<i>ccdB</i> Survival 2 T1 ^R	<i>E. coli</i> CcdB-resistant cloning strain (genotype unknown)	Invitrogen
DB3.1	<i>E. coli</i> CcdB-resistant cloning strain (<i>gyrA462</i>)	Invitrogen
MFDpir $\Delta hsdR$	<i>E. coli</i> MFDpir $\Delta hsdR$; conjugation donor	C. M. Tang (Oxford)
MG1655	<i>E. coli</i> K-12 MG1655 wild type	[45]
PAO1	<i>P. aeruginosa</i> PAO1 wild type	[64]

Supplementary Table 2: Plasmids used in this study.

Plasmid ID	Construction/purpose	Source
pAggR	pGM101 _{neo} containing entire <i>aggR</i> locus	This study
pBAD33	Arabinose-inducible vector for toxicity assays	[53]
pBAD33:: <i>ccdB</i> ₁₁₀	pBAD33 encoding CcdB ₁₁₀	This study
pBAD33:: <i>ccdB</i> ₁₀₁	pBAD33 encoding CcdB ₁₀₁	This study
pBAD33:: <i>ccdB</i> _{M90T}	pBAD33 encoding CcdB _{M90T}	This study
pBAD33:: <i>relE1</i>	pBAD33 encoding RelE/ParE 1	This study
pBAD33:: <i>relE2</i>	pBAD33 encoding RelE/ParE 2	This study
pBAD33:: <i>vapC</i> ₁₄₂	pBAD33 encoding VapC ₁₄₂	This study
pBAD33:: <i>vapC</i> ₁₃₃	pBAD33 encoding VapC ₁₃₃	This study
pCcdB	pGM101 _{neo} containing entire <i>ccdAB</i> locus	This study
pCONJ5K	Mobilisable vector for mutagenesis	[34]
pGM101	Promoterless vector for antitoxicity assays	[30]
pGM101 _{neo}	Neomycin-resistant variant of pGM101	This study
pGM101 _{neo} :: <i>ccdA</i>	pGM101 _{neo} containing <i>ccdA</i> plus its promoter	This study
pIB279	<i>sacB</i> -Neo ^R cassette for pSTAB vectors	[58]
pMW_O104	pSTAB with FII/FIB replicons from pAA ₁₀₇₀	This study
pMW_O104:: <i>ccdAB</i>	pMW_O104 containing entire <i>ccdAB</i> locus	This study
pUC19	High-copy cloning vector	New England Biolabs

Supplementary Table 3: Oligonucleotide primers used in this study.

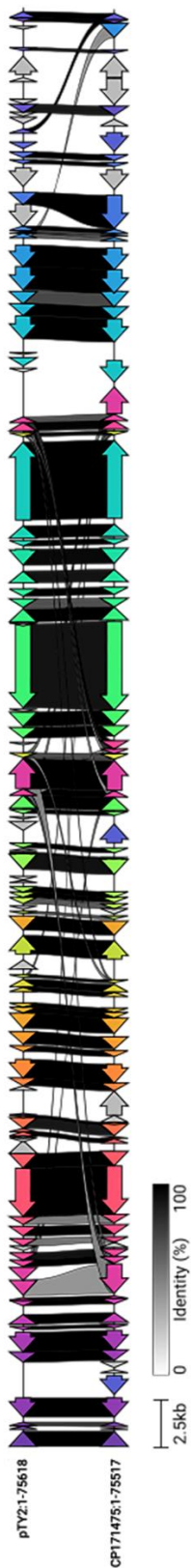
Primer ID	Sequence (5'-3')	Target
GMntu106	gcgcacccagaggacatcttatattcccagaacatcaggttaatg	<i>ccdAB</i> for pSTAB
GMntu107	gtgatgggttaaaaaggatccggatctgccggaatgg	
GMntu112	gcgcacccagaggacatcgatccttttaacccatcacatatacctgccgttcac	<i>sacB-NeoR</i>
GMntu113	gttaaaaaggatcgatgtccctctgggatgcgctccggatgaatatgatgatc	pAA ₁₀₇₀ replicon
GMntu114	caggacgacgaggcttgacacctcataatcggtagtgatgctgttttctgg	
GMntu115	gcatacactaccgattatgaaggtgcaagcctcgctcctggccggaccacgctatctg	<i>sacB-NeoR</i>
GMntu132	ccatttcggcgagatccggatccttttaacccatcacatatacctgccgttcac	pMW_O104 for
GMntu133	ctggggaatataagatgtccctctgggatgcgctccggatgaatatgatgatc	<i>ccdAB</i> insertion
MS123	cgaagcggcatgcatttacg	pGM101 for
MS124	ccttcgcgcgcgaattgatc	complementation
MS125	gatcaattcgcgcgcgaaggactgggctgcaattaag	<i>aggR</i> for
MS126	cgtaaatgcatgccgcttcgctcattggcttttaaaataagtc	complementation
MS127	gatcaattcgcgcgcgaaggcttcctcagtggtgtacac	<i>ccdAB</i> for
MS128	cgtaaatgcatgccgcttcgcttatattcccagaacatcag	complementation
RWntu011	ccgggtaccgagctcgaattc	pBAD33
RWntu012	ggatcctctagagtcgac	
RWntu013	aattcgagctcggtagccggcgacgaaggaagatttgac	<i>vapC</i> ₁₄₂
RWntu014	aggctgactctagaggatccttactcaccagtccttc	
RWntu015	aattcgagctcggtagccgggacccgaacgaagacctatag	<i>vapC</i> ₁₃₃
RWntu016	aggctgactctagaggatccttactcaccagtccttc	
RWntu017	aattcgagctcggtagccgggtattgaaatgaacggctc	<i>ccdB</i> ₁₁₀
RWntu018	aggctgactctagaggatccttatattcccagaacatcag	
RWntu028	agaaacgcaaaaaggccatc	pGM101 (to make pGM101 _{neo})
RWntu029	ctgtcagaccaagttactc	
RWntu030	gatggccttttgcgtttctcttcacgctgccgcaagc	<i>neoR</i>
RWntu031	gagtaaaacttggtctgacagaggcggcggtggaatcga	
RWntu040	tcgcgcgcgaaggcggatctgccggaatgg	<i>ccdA</i>
RWntu041	gcatgccgcttcgtcaccagtcctctgtctc	
RWntu074	aggctgactctagaggatccttatattcccagaacatcagg	<i>ccdB</i> ₁₀₁
RWntu075	aattcgagctcggtagccggttgccgatgagaacagg	
RWntu081	aattcgagctcggtagccggaatgaacggctcttttg	<i>ccdB</i> _{M90T}
RWntu082	aggctgactctagaggatccttatattcccagaacatcag	
RWntu088	aattcgagctcggtagccggaactgacaatccgttacg	<i>relE/parE</i> 1
RWntu089	aggctgactctagaggatccctacgttttctcgtgtg	
RWntu090	aattcgagctcggtagccggaagctggcgcgagggtt	<i>relE/parE</i> 2
RWntu091	aggctgactctagaggatcctcagtgaaatgacggctggcatc	
RWntu106	tcgtttgaaatcccgttcg	<i>dnaA</i> (ddPCR)
RWntu107	cgttttcgtcggccttttc	
RWntu108	agccgatccgttaaaagcac	<i>fliC</i> (ddPCR)
RWntu109	tggtggtgtgttcagggtg	
RWntu110	tgtaacgaacgtcgcgaag	<i>hns</i> (ddPCR)
RWntu111	attgctgcagcttacgagtg	
RWntu112	gctgtaggaccacttattagc	<i>aggA</i> (ddPCR)
RWntu113	tcctccacaaaactgttggtg	
RWntu114	atatcgggtggtcatcatgcg	<i>ccdB</i> (ddPCR)
RWntu115	aagtctcccgtgaactttaccc	
RWntu116	gcaatagccaattgcacat	<i>wzy</i> (ddPCR)
RWntu117	cccggggcaattatcattaa	
RWntu140	ggtaccggggatcctctag	pUC19
RWntu141	gagctcgaattcactggcc	
RWntu142	cggccagtgaaatcgagctcagtggtgcaaaaaatggtg	<i>aggR</i> downstream
RWntu143	cagcctacacttgactattttaaaagccaatg	

Supplementary Table 3 cont.: Oligonucleotide primers used in this study.

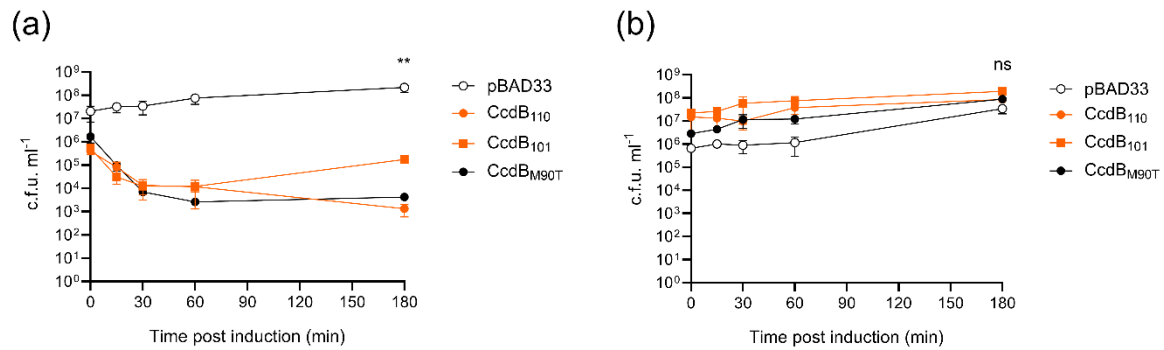
Primer ID	Sequence (5'-3')	Target
RWntu144	aataagtcaagtgtaggctggagctgcttc	<i>cat</i> (Δ <i>aggR</i>)
RWntu145	tgataaagacatgggaattagccatgggtcc	
RWntu146	taattcccatgtctttatcaggcaactctgc	<i>aggR</i> upstream
RWntu147	ctagaggatccccgggtaccggcgcgcatcctgtatattattg	
RWntu148	cagcctacacctgatgttctggggaatataaatgtc	<i>ccdB</i> downstream
RWntu149	cggccagtgaattcgagctcgcgctttctgtcctgc	
RWntu150	tggtgaaatgatgggaattagccatgggtcc	<i>cat</i> (Δ <i>ccdB</i>)
RWntu151	agaacatcagggtgtaggctggagctgcttc	
RWntu152	ctagaggatccccgggtaccctccctgacctgtgatgc	<i>ccdB</i> upstream
RWntu153	taattcccatcattcaccagtcctgttc	
RWntu154	aacgacggccagtggttaaaaggcgcgcatcctgtatattattg	<i>aggR</i> (deletion)
RWntu155	agcaggaaacagctatgacgcagtggttgcaaaaatgggtgg	
RWntu156	cgtcatagctgttcctg	pCONJ5K
RWntu157	tttaaactggccgtcg	
RWntu158	agcaggaaacagctatgacggcgctttctgtcctgc	<i>ccdB</i> (deletion)
RWntu159	aacgacggccagtggttaaaactccctgacctgtgatgc	

Supplementary Table 4: ddPCR master mix.

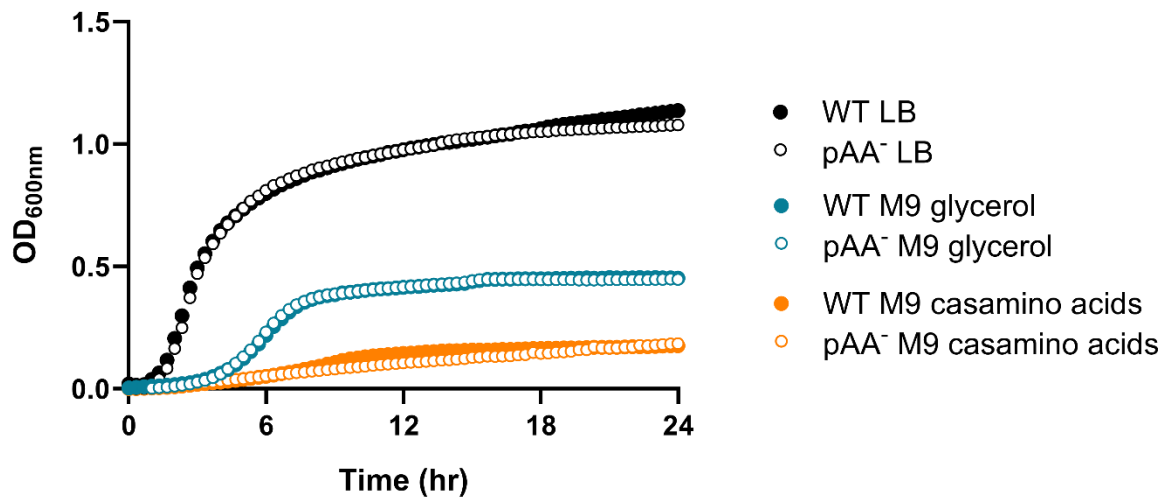
Component	Concentration or quantity per 20 μ l reaction
2x ddPCR Supermix for Probes (No dUTP)	10 μ l
Target primer/probe mix (FAM)	900 nM primers/250 nM probes
Reference primer/probe mix (HEX)	900 nM primers/250 nM probes
DNA	50 ng



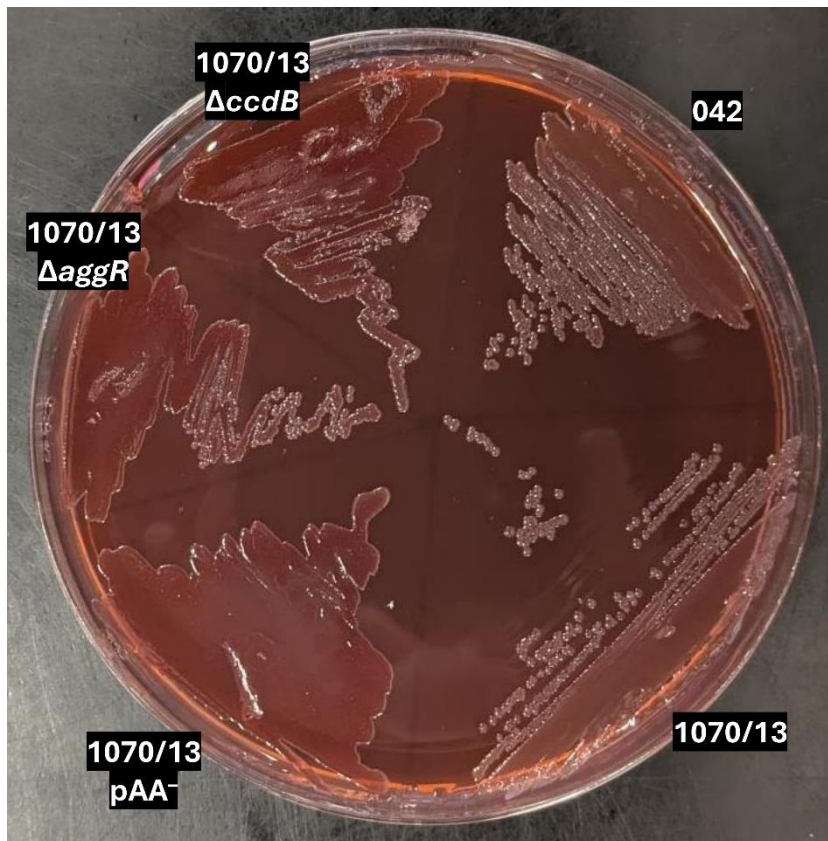
Supplementary Figure 1: Comparative analysis of linear plasmid maps pTY2 and pAA₁₀₇₀ (CP171475). Colours represent homologous CDSs. Homologous regions shared between the two plasmids are shaded in grey according to percentage nucleotide identity greater than 70 %. Image produced using clinker gene cluster comparison [42] (<https://cagecat.bioinformatics.nl/>).



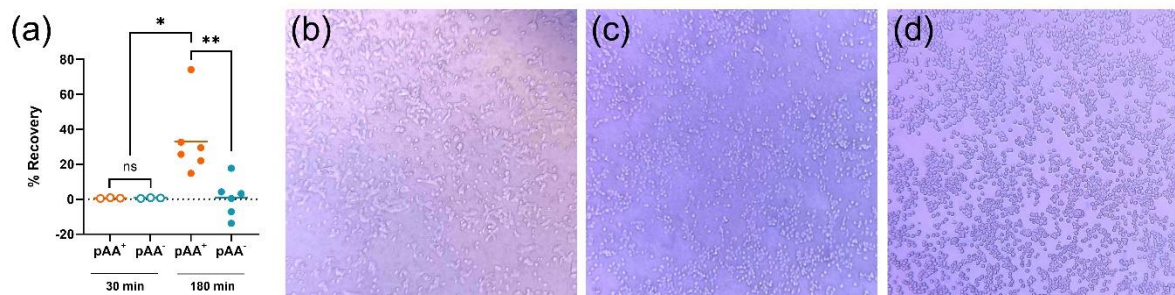
Supplementary Figure 2: Toxicity of CcdB from pAA₁₀₇₀ in CcdB-resistant cloning strains. Mean bacterial viability after induction of toxin production from pBAD33; (a) in *E. coli ccdB* Survival 2 T1^R; (b) in *E. coli* gyrase mutant DB3.1. Empty vector control in each experiment is indicated as pBAD33. Error bars show standard error of the mean. Statistical analysis by two-way ANOVA with Dunnett's multiple comparison tests: ns, not significant ($P > 0.05$); ** $P < 0.01$ (from $n = 3$ biological replicates).



Supplementary Figure 3: Growth of O104:H4 strain 1070/13 (WT) and its plasmid-free derivative (pAA⁻) in various laboratory media. OD_{600nm} measured every 20 minutes at 37 °C with aeration in 24-well plate. Mean of two biological replicates shown (from six technical replicates).



Supplementary Figure 4: Growth of O104:H4 strain 1070/13 and its derivatives on YESCA medium supplemented with Congo red dye. Plate incubated at 37 °C.



Supplementary Figure 5: Effect of pAA on adherence of O104:H4 strain 1070/13 to HT-29 cells. (a) Per cent recovery of bacterial cells (normalised to inoculum) after incubation for either 30 min (empty circles) or 180 min (solid circles) with HT-29 cells. Each point is one biological replicate. Solid lines show the mean. Statistical analysis by one-way ANOVA with Tukey's multiple comparison tests: ns, not significant ($P \geq 0.05$); * $P < 0.05$; ** $P < 0.01$ (from $n \geq 3$ biological replicates). (b-d) Light microscopy at 40x magnification of HT-29 cells at 180 min post-infection with either (b) 1070/13, (c) 1070/13 pAA⁻ or (d) no bacteria.