

Table S3. Strains, plasmids, and primers used in the study

Strains	Description	Source/comment
EPEC	EPEC, E2348/69, streptomycin-resistant	
EHEC O157:H7	EHEC 86-24, Shiga toxin positive	
<i>Citrobacter rodentium</i>	<i>C. rodentium</i> DBS100	ATCC 51459
EPEC $\Delta cesT$	<i>cesT</i> deletion mutant	
EPEC $\Delta sepD$	<i>sepD</i> deletion mutant	
EPEC $\Delta sepD\Delta cesT$	Double mutant derived from $\Delta sepD$	
EPEC <i>cesTY152F</i>	Expresses CesT(Y152F) from the EPEC chromosomal <i>cesT</i> locus	This study
EPEC <i>cesTY153F</i>	Expresses CesT(Y153F) from the EPEC chromosomal <i>cesT</i> locus	This study
EPEC <i>cesTY152F, Y153F</i>	Expresses CesT(Y152F, Y153F) from the EPEC chromosomal <i>cesT</i> locus	This study
<i>C. rodentium cesTY152F</i>	Expresses CesT(Y152F) from the <i>C. rodentium</i> chromosomal <i>cesT</i> locus	This study
<i>C. rodentium cesTY153F</i>	Expresses CesT(Y153F) from the <i>C. rodentium</i> chromosomal <i>cesT</i> locus	This study
<i>C. rodentium cesTY152F, Y153F</i>	Expresses CesT(Y152F, Y153F) from the <i>C. rodentium</i> chromosomal <i>cesT</i> locus	This study
DH5 α	<i>E. coli</i> strain used for cloning	
DH5 $\alpha\lambda pir$	<i>E. coli</i> strain used for cloning, permissive for pRE112 replication	
BL21(λ DE3)	<i>E. coli</i> host for protein overexpression	
EPEC <i>tir::tir-blaM</i>	Merodiploid chromosomal construct for expression of Tir-TEM-1 built into EPEC	This study
EPEC <i>espF::espF-blaM</i>	Merodiploid chromosomal construct for expression of EspF-TEM-1 built into EPEC	This study
EPEC <i>nleA::nleA-blaM</i>	Merodiploid chromosomal construct for expression of NleA-TEM-1 built into EPEC	This study
EPEC <i>nleH1::nleH1-blaM</i>	Merodiploid chromosomal construct for expression of NleH1-TEM-1 built into EPEC	This study
EPEC <i>nleH2::nleH2-blaM</i>	Merodiploid chromosomal construct for expression of NleH2-TEM-1 built into EPEC	This study
$\Delta cesT$ <i>tir::tir-blaM</i>	Merodiploid chromosomal construct for expression of Tir-TEM-1 built into EPEC $\Delta cesT$	This study
$\Delta cesT$ <i>espF::espF-blaM</i>	Merodiploid chromosomal construct for expression of EspF-TEM-1 built into EPEC $\Delta cesT$	This study
$\Delta cesT$ <i>nleA::nleA-blaM</i>	Merodiploid chromosomal construct for expression of NleA-TEM-1 built into EPEC $\Delta cesT$	This study
$\Delta cesT$ <i>nleH1::nleH1-blaM</i>	Merodiploid chromosomal construct for expression of NleH1-TEM-1 built into EPEC $\Delta cesT$	This study
$\Delta cesT$ <i>nleH2::nleH2-blaM</i>	Merodiploid chromosomal construct for expression of NleH2-TEM-1 built into EPEC $\Delta cesT$	This study
<i>cesT</i> (Y152F) <i>tir::tir-blaM</i>	Merodiploid chromosomal construct for expression of Tir-TEM-1 built into EPEC <i>cesT</i> (Y152F)	This study

<i>cesT</i> (Y152F) <i>espF::espF-blaM</i>	Merodiploid chromosomal construct for expression of EspF-TEM-1 built into EPEC <i>cesT</i> (Y152F)	This study
<i>cesT</i> (Y152F) <i>nleA::nleA-blaM</i>	Merodiploid chromosomal construct for expression of NleA-TEM-1 built into EPEC <i>cesT</i> (Y152F)	This study
<i>cesT</i> (Y152F) <i>nleH1::nleH1-blaM</i>	Merodiploid chromosomal construct for expression of NleH1-TEM-1 built into EPEC <i>cesT</i> (Y152F)	This study
<i>cesT</i> (Y152F) <i>nleH2::nleH2-blaM</i>	Merodiploid chromosomal construct for expression of NleH2-TEM-1 built into EPEC <i>cesT</i> (Y152F)	This study
<i>cesT</i> (Y153F) <i>tir::tir-blaM</i>	Merodiploid chromosomal construct for expression of Tir-TEM-1 built into EPEC <i>cesT</i> (Y153F)	This study
<i>cesT</i> (Y153F) <i>espF::espF-blaM</i>	Merodiploid chromosomal construct for expression of EspF-TEM-1 built into EPEC <i>cesT</i> (Y153F)	This study
<i>cesT</i> (Y153F) <i>nleA::nleA-blaM</i>	Merodiploid chromosomal construct for expression of NleA-TEM-1 built into EPEC <i>cesT</i> (Y153F)	This study
<i>cesT</i> (Y153F) <i>nleH1::nleH1-blaM</i>	Merodiploid chromosomal construct for expression of NleH1-TEM-1 built into EPEC <i>cesT</i> (Y153F)	This study
<i>cesT</i> (Y153F) <i>nleH2::nleH2-blaM</i>	Merodiploid chromosomal construct for expression of NleH2-TEM-1 built into EPEC <i>cesT</i> (Y153F)	This study
<i>cesT</i> (Y152F, Y153F) <i>tir::tir-blaM</i>	Merodiploid chromosomal construct for expression of Tir-TEM-1 built into EPEC <i>cesT</i> (Y152F, Y153F)	This study
<i>cesT</i> (Y152F, Y153F) <i>espF::espF-blaM</i>	Merodiploid chromosomal construct for expression of EspF-TEM-1 built into EPEC <i>cesT</i> (Y152F, Y153F)	This study
<i>cesT</i> (Y152F, Y153F) <i>nleA::nleA-blaM</i>	Merodiploid chromosomal construct for expression of NleA-TEM-1 built into EPEC <i>cesT</i> (Y152F, Y153F)	This study
<i>cesT</i> (Y152F, Y153F) <i>nleH1::nleH1-blaM</i>	Merodiploid chromosomal construct for expression of NleH1-TEM-1 built into EPEC <i>cesT</i> (Y152F, Y153F)	This study
<i>cesT</i> (Y152F, Y153F) <i>nleH2::nleH2-blaM</i>	Merodiploid chromosomal construct for expression of NleH2-TEM-1 built into EPEC <i>cesT</i> (Y152F, Y153F)	This study
Plasmids	Description	Source/comment
pBluescript	High copy cloning vector	Stratagene
pRE112	Suicide plasmid for allelic exchange	
pEPcesT	Plasmid containing EPEC <i>tir-cesT-eae</i> locus	This study
pCRcesT	Plasmid containing <i>C. rodentium tir-cesT-eae</i> locus	This study
pFLAG-CTC	Expression vector for expression of ORF-FLAG fusion constructs from the <i>tac</i> promoter	Sigma
pCesT-FLAG	EPEC <i>cesT</i> cloned as a HindIII/KpnI fragment into the pFLAG-CTC expression	Ref. 30

	vector, expresses CesT-FLAG from the <i>tac</i> promoter	
pCesT(Y152F/Y153F)-FLAG	EPEC <i>cesT</i> (Y152F/Y153F) cloned as a HindIII/KpnI fragment into the pFLAG-CTC expression vector, expresses CesT(Y152F/Y153F)-FLAG from the <i>tac</i> promoter	This study
pCesT(Y152F)-FLAG	EPEC <i>cesT</i> (Y152F) cloned as a HindIII/KpnI fragment into the pFLAG-CTC expression vector, expresses CesT(Y152F)-FLAG from the <i>tac</i> promoter	This study
pCesT(Y153F)-FLAG	EPEC <i>cesT</i> (Y153F) cloned as a HindIII/KpnI fragment into the pFLAG-CTC expression vector, expresses CesT(Y153F)-FLAG from the <i>tac</i> promoter	This study
pSec10	Low-copy number vector	Ref. 32
pAMH275	Expression of EHEC <i>cesT</i> from pSec10	This study
pAMH276	Expression of EHEC <i>cesT</i> Y152F from pSec10	This study
pAMH277	Expression of EHEC <i>cesT</i> Y153F from pSec10	This study
pAMH278	Expression of EHEC <i>cesT</i> Y152F, Y153F from pSec10	This study
pAMH279	Expression of EHEC <i>cesT</i> Y152D from pSec10	This study
pAMH280	Expression of EHEC <i>cesT</i> Y153D from pSec10	This study
pAMH281	Expression of EHEC <i>cesT</i> Y152D, Y153D from pSec10	This study
pAT113	Monocopy expression vector for GFP; used to transform chromosomal EPEC <i>cesT</i> variant strains for fluorescence microscopy	Ref. 86
pNleA-FLAG	pACYC184 with cloned <i>nleA</i> allele, expresses NleA-FLAG from the <i>nleA</i> promoter	Ref. 22
pCX442	pCX391 derivative; conjugative suicide plasmid that encodes <i>tir-blaM</i> ; received from Ilan Rosenshine	Ref. 21
pCX446	pCX391 derivative; conjugative suicide plasmid that encodes <i>espF-blaM</i> ; received from Ilan Rosenshine	Ref 21

Primers used in this study

Primer	Sequence (5'-3')	Purpose
NT241	CCGCTAGCATGTCATCAAGATCTGAACTT	His-CesT constructs
CesTRev	CCGAGCTCGTAAATTAATACTAATAAATAAGTTTTA	His-CesT constructs

NT379	AAGAGCTCCTCGGAATAGTCTATCGGCTCA	pEPcesT
NT380	TATCGCTTGAGCTAATTCCTC	pEPcesT
NT381	AAGAGCTCTCCTAACTATAGCGTTATACAGC	pCRcesT
NT382	TTTTCATATGTTCAATTTCTAATGTTATTCC	pCRcesT
CesT/For/FLAG	CCCAAGCTTTCATCAAGATCTGAACTTTTA	CesT-FLAG
CR003-152/153	CCGGTACCTCTTCCGGCGAAAAAATGTTTATTATCGC	CesT(Y152F/Y153F)
CR004-152	CCGGTACCTCTTCCGGCGTAAAAAATGTTTATTATC	CesT(Y152F)
CR005-153	CCGGTACCTCTTCCGGCAAATAATGTTTATTATC	CesT(Y153F)
AH1094	CATGTGTCGACGCGTTACAGCAACCTCTTGATATGAA	EHEC <i>LEE5</i> regulatory region FW
AH1095	CATCGACCAAATAAATCACAATCTGATTTTTGATG	EHEC <i>LEE5</i> regulatory region BW
AH1096	CCATAATCATTTTATTTAGAGGGAGGGA	EHEC <i>cesT</i> FW
AH1265	CATCGAATTCGGATTTTATCTTCCGGCGTAAAAATGT TTATTGTCG	EHEC <i>cesT</i> BW
AH1259	CATCGAATTCGGATTTTATCTTCCGGCGTAATCA TGTTTATTGTCG	EHEC <i>cesT</i> Y152D BW
AH1260	CATCGAATTCGGATTTTATCTTCCGGCGTCATAA TGTTTATTGTCG	EHEC <i>cesT</i> Y153D BW
AH1261	CATCGAATTCGGATTTTATCTTCCGGCATCGTCA TGTTTATTGTCGCT	EHEC <i>cesT</i> Y152D/Y153D BW
AH1280	CATCGAATTCGGATTTTATCTTCCGGCGTAAAAATGT TTATTGTCG	EHEC <i>cesT</i> Y152F BW
AH1281	CATCGAATTCGGATTTTATCTTCCGGCGAAATAATGT TTATTGTCG	EHEC <i>cesT</i> Y153F BW
AH1282	CATCGAATTCGGATTTTATCTTCCGGCGAAAAAATGT TTATTGTCGCT	EHEC <i>cesT</i> Y152F/Y153F BW
CR-NleA-fwd	GCGGTACCATGAACATTCAACCGATCGTAAC	<i>nleA-blaM</i>
CR-NleA-rev	GCGAATTCCTGACTCTTGTCTTGGATTATATC	<i>nleA-blaM</i>
CR-NleH1-fwd	GCGGTACCATGCTATCACCATCTTCTGTAAA	<i>nleH1-blaM</i>
CR-NleH1-rev	GCACAATTGCCAATTTACTTAATACCACACTAATAAG	<i>nleH1-blaM</i>
CR-NleH2-fwd	GCGGTACCATGTTATCGCCCTCTTCTATAA	<i>nleH2-blaM</i>
CR-NleH2-rev	GCACAATTGCCTATCTTACTTAATACTACACTAATAAG	<i>nleH2-blaM</i>
CR-NleA-Bla-seqF	GCATGCAAGAGAAGGTGCCATTTTCC	<i>nleA-blaM</i> sequencing
CR-NleH1-Bla-seqF	CGATACAACAGAGACAAATGTCTTATATGATCGCACG	<i>nleH1-blaM</i> sequencing

CR-NleH2-Bla-seqF	CCATCATTACCAGCACAAAGCTGAACAGGC	<i>nleH2-blaM</i> sequencing
CR-Tir-Bla-seqF	GGCAAGGTATCCAAAGTACTTATGCGC	<i>Tir-blaM</i> sequencing
CR-EspF-Bla-seqF	CGAAAAGTCTTGATACTTCAGGTCTTAAGCCAAGCC	<i>espF-blaM</i> sequencing