Table S1.
Strains, plasmids and oligonucleotides used in this study.

STRAINS

Strains	description and genotype	source
Enteroaggregative *E. coli*		
17-2	WT enteroaggregative *Escherichia coli*	Arlette Darfeuille-Michaud
17-2Δ*hcp*	17-2 deleted of the *sci-1 hcp* gene	[10]
17-2Δ*sci-1*	17-2 deleted of the whole *sci-1* gene cluster	This study
17-2Δ*tssB1*	17-2 deleted of the *sci-1 tssB* gene	This study
17-2Δ*tssC1*	17-2 deleted of the *sci-1 tssC* gene	This study
17-2Δ*tssBC1*	17-2 deleted of the *sci-1 tssB* and *tssC* genes	This study
17-2Δ*vgrG1*	17-2 deleted of the *sci-1 vgrG* gene	This study
E. coli K12		
DH5α	F-, Δ(*argF-lac*)U169, *phoA*, *supE44*, Δ(lacZ)M15, *relA*, *endA*, *thi*, *hsdR*	Laboratory collection
W3110	F-, lambda- IN(*rrnD-rrnE*)1 *rph*-1	Laboratory collection

PLASMIDS

pUC12	empty vector, pColE1 origin, P*lac*, Amp^R	[22]
pUC-Hcp1-FLAG	*sci-1 hcp* (*hcp1*) cloned into pUC12, C-terminal FLAG epitope	[10]
pUC-Hcp2-FLAG	*sci-2 hcp* (*hcp2*) cloned into pUC12, C-terminal FLAG epitope	This study
pUC-Hcp1-FLAG N115C	code for Hcp1 harbouring the N115C mutation	This study
pUC-Hcp1-FLAG C38S	code for Hcp1 harbouring the C38S mutation	This study
pUC-Hcp1-FLAG C38S Q24C	code for Hcp1 harbouring the C38S and Q24C mutations	This study
pUC-Hcp1-FLAG C38S G48C	code for Hcp1 harbouring the C38S and G48C mutations	This study
pUC-Hcp1-FLAG C38S A95C	code for Hcp1 harbouring the C38S and A95C mutations	This study
pUC-Hcp1-FLAG C38S G96C	code for Hcp1 harbouring the C38S and G96C mutations	This study
pUC-Hcp1-FLAG C38S N115C	code for Hcp1 harbouring the C38S and N115C mutations	This study
pUC-Hcp1-FLAG C38S S158C	code for Hcp1 harbouring the C38S and S158C mutations	This study
pUC-Hcp1-FLAG C38S Q24C A95C	code for Hcp1 harbouring the C38S, Q24C and A95C mutations	This study
pUC-Hcp1-FLAG C38S G96C S158C	code for Hcp1 harbouring the C38S, G96C and S158C mutations	This study
pUC-Hcp1-FLAG S158W	code for Hcp1 harbouring the S158W mutation	This study
pT18-FLAG	Bacterial Two Hybrid (BACTH) vector	[23]
pT18-Hcp1	*hcp1* cloned downstream the T18 coding sequence in p-T18-FLAG	This study
pHcp1-T18	*hcp1* cloned upstream the T18 coding sequence in p-T18-FLAG	This study
pT18-TssB1	*tssB1* cloned downstream the T18 coding sequence in p-T18-FLAG	This study
pTssB1-T18	*tssB1* cloned upstream the T18 coding sequence in p-T18-FLAG	This study
pT18-TssC1	*tssC1* cloned downstream the T18 coding sequence in p-T18-FLAG	This study
pTssC1-T18	*tssC1* cloned upstream the T18 coding sequence in p-T18-FLAG	This study
pT18-Pal	*pal* cloned downstream the T18 coding sequence in p-T18-FLAG	[23]
pT25-FLAG	Bacterial Two Hybrid (BACTH) vector	[23]
pT25-Hcp1	*hcp1* cloned downstream the T25 coding sequence in p-T25-FLAG	This study
pHcp1-T25	*hcp1* cloned upstream the T25 coding sequence in p-T25-FLAG	This study
pT25-TssB1	*tssB1* cloned downstream the T25 coding sequence in p-T25-FLAG	This study
pTssB1-T25	*tssB1* cloned upstream the T25 coding sequence in p-T25-FLAG	This study

pT25-TssC1	*tssC1* cloned downstream the T25 coding sequence in p-T25-FLAG	This study
pTssC1-T25	*tssC1* cloned upstream the T25 coding sequence in p-T25-FLAG	This study
pTolB-T25	*tolB* cloned upstream the T25 coding sequence in p-T25-FLAG	[23]
pBAD33	empty vector, pACYC184 origin, P*ara*, *araC*, Cm^R	[24]
pBAD33-TssB1-VSV-G	*tssB1* cloned into pBAD33, C-terminal VSV-G epitope	This study
pBAD33-TssB2-VSV-G	*tssB2* cloned into pBAD33, C-terminal VSV-G epitope	This study
pBAD33-TssB1-sfGFP	*sfgfp* fused in-frame to *tssB1* (separated by a 3×Ala 3×Gly linker) in pBAD33-TssB1-VSV-G	This study
pBAD33-VgrG-VSV-G	*sci-1 vgrG* (*vgrG1*) cloned into pBAD33, C-terminal VSV-G epitope	This study
pOK12	empty vector, P15A origin, P*lac*, Kan^R	[22]
pOK12-TssB1-HA	*sci-1 tssB* (*tssB1*) cloned into pOK12, C-terminal HA epitope	M.-S. Aschtgen

OLIGONUCLEOTIDES

For strain construction [a]

5−Δ*hcp1*	TCCCCTGCGCCGGAAGAGGGCGCATCAGAAAACATAACGGAGTAATTTTTTGTGTAGGCTGGAGCTGCTTCG
3-Δ*hcp1*	TGAAGAAAAAATAAAAATGACGGACAGGATGCCCTGTCCGTCCGGCAGAACATATGAATATCCTCCTTAGTTCC
5−Δ*sci-1*	GGAGGCATCTGCGGTGATGGAACCCCTGAGATGCAGGTTTCACAGGAGAGAGCCTGTGTAGGCTGGAGCTGCTTCG
3-Δ*sci-1*	CATATATCCATCGCTGTTTATTTATTTCTGTTCTGCCCTCCCTGCTCCGGCCATATGAATATCCTCCTTAGTTC
5−Δ*tssB1*	GCATCTGCGGTGATGGAACCCCTGAGATGCAGGTTTCACAGGAGAGAGCCGTGTAGGCTGGAGCTGCTTCG
3-Δ*tssB1*	TTCTTTTCTTTCTGTACAGACATCAGCATTTTCTCTCGTAATCCGTTAAACATATGAATATCCTCCTTAGTTCC
5−Δ*tssC1*	TGCGTGCACTGGTGCCGGAAAAGGCGTGATTTAACGGATTACGAGAGAAAGTGTAGGCTGGAGCTGCTTCG
3-Δ*tssC1*	GCCCCGTCTTCCCATAATGGGCGATAAATCTTCATTTCCCGACACCTGCCGCATATGAATATCCTCCTTAGTTCC
5−Δ*vgrG*	TACCCTTTCAGAGCTGTTCGTGGAGTGATAAATCATGAATCTCACTGAGTGTAGGCTGGAGCTGCTTCG
3-Δ*vgrG*	GGTTTTATGGGTGGCATCTGTTACGTCATATCCCTGATATTTTGTCATTCCATATGAATATCCTCCTTAGTTCC

For plasmid construction [b, c, d]

5pUC-Hcp2-FLAG	GGAAACAGCTATGACCATGATTACGAATATGGTCGGAGTTATGAGTA
3pUC-Hcp2-FLAG	AAGAGGATCCCCGGGCGAGCTCGATTA**TTTATCATACTCATCTTTATAATC**TACAAGAGCCT CTTTATATAAG
5pBAD-TssB1-VSV-G	CTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGTATTACACCATGAGCAGTT CGTTTCAGAATGAAATCCCG
3pBAD-TssB1-VSV-G	GGTCGACTCTAGAGGATCCCCGGGTACCTTA**TTTTCCTAATCTATTCATTTCAATATCTGTA TA**CGCCTTTTCCGGCACCAGTGCAC
5pBAD-TssB2-VSV-G	CTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGTATTACACCATGAGCAATA CACAACATAAACTTGATAAAAC
3pBAD-TssB2-VSV-G	GGTCGACTCTAGAGGATCCCCGGGTACCTTA**TTTTCCTAATCTATTCATTTCAATATCTGTA TA**TTCCTTACTTTCATCGTTTTCAGGATTG

5pBAD-TssB1-sfGFP	CACTGGTGCCGGAAAAGGCG*GCAGCGGCCGGCGGAGGG*TCTAAAGGTGAAGAACTGTTCACCG
3pBAD-TssB1-sfGFP	CGGGTACCTTATTTTCCTAATCTATTCATTTCAATATCTGTATAGGAAACAGCTATGACCATGATTACG
5pBAD-VgrG1-VSV-G	CTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGTATTACACCATGAATCTCACTGAC TCCCTGCAAAATGTTTTATCC
3pBAD-VgrG1-VSV-G	GGTCGACTCTAGAGGATCCCCGGGTACCTTA**TTTTCCTAATCTATTCATTTCAATATCTGTATA**TTC TGTTTCTCCATGAATTTTTACCTTCCCAAACTC
T25T18C-5-Hcp1	CGGATAACAATTTCACACAGGAAACAGCTATGACCATGGCAATTCCAGTTTATCTGTGGCTGAAAG ATG
T18C-3-Hcp1	CCTCGCTGGCGGCTAAGCTTGGCGTAATCGCGGTGGTACGCTCACTCCATGCG
T25C-3-Hcp1	GTTTGCGTAACCAGCCTGATGCGATTGCTGCGCGGTGGTACGCTCACTCCATGCG
T25T18N-3-Hcp1	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACGCGGTGGTACGCTCACTCCATGCG
T18N-5-Hcp1	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGGCAATTCCAGTTTATCTGTGGCTGAAAGATG
T25N-5-Hcp1	GGCGGGCTGCAGATTATAAAGATGACGATGACAAGGCAATTCCAGTTTATCTGTGGCTGAAAGATG
T25T18C-5-TssB1	CGGATAACAATTTCACACAGGAAACAGCTATGACCATGAGCAGTTCGTTTCAGAATGAAATCCCGA AAGCAAG
T18C-3-TssB1	CCTCGCTGGCGGCTAAGCTTGGCGTAATCGCCTTTTCCGGCACCAGTGCACGC
T25C-3-TssB1	GTTTGCGTAACCAGCCTGATGCGATTGCTGCGCCTTTTCCGGCACCAGTGCACGC
T25T18N-3-TssB1	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACGCCTTTTCCGGCACCAGTGCACGC
T18N-5-TssB1	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGAGCAGTTCGTTTCAGAATGAAATCCCGAAAG CAAG
T25N-5-TssB1	GGCGGGCTGCAGATTATAAAGATGACGATGACAAGAGCAGTTCGTTTCAGAATGAAATCCCGAAAGC AAG
T25T18C-5-TssC1	CGGATAACAATTTCACACAGGAAACAGCTATGACCATGCTGATGTCTGTACAGAAAGAAAAGAACGT TGCAGAGAGCG
T18C-3-TssC1	CCTCGCTGGCGGCTAAGCTTGGCGTAATCGCTTTTGCCTTCGGCATCTGCGAAACCAGTG
T25C-3-TssC1	GTTTGCGTAACCAGCCTGATGCGATTGCTGCGCTTTTGCCTTCGGCATCTGCGAAACCAGTG
T25T18N-3-TssC1	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACGCTTTTGCCTTCGGCATCTGCGAA ACCAGTG
T18N-5-TssC1	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGCTGATGTCTGTACAGAAAGAAAAGAACGTT GCAGAGAGCG
T25N-5-TssC1	GGCGGGCTGCAGATTATAAAGATGACGATGACAAGCTGATGTCTGTACAGAAAGAAAAGAACGTTG CAGAGAGCG

For site-directed mutagenesis [e]

A-Hcp1-N115C	CGTGAAGGTGGTGAAGGTTTGCCCGGTCATGCACGACATC
B-Hcp1-N115C	GATGTCGTGCATGACCGGGCAAACCTTCACCACCTTCACG
A-Hcp1-S158C	CATTCATTCCGACGCATGGTGTGAGCGTACCACCGCGTAA
B-Hcp1-S158C	TTACGCGGTGGTACGCTCACACCATGCGTCGGAATGAATG
A-Hcp1-Q24C	CAAAGGGTCCGTGGATGTTTGCGACCGTGAGGGCAGCATTG
B-Hcp1-Q24C	CAATGCTGCCCTCACGGTCGCAAACATCCACGGACCCTTTG
A-Hcp1-G48C	TCCCGACGGATAACAATACCTGTAAGCTGACCGGTACCCG
B-Hcp1-G48C	CGGGTACCGGTCAGCTTACAGGTATTGTTATCCGTCGGGA
A-Hcp1-A95C	GTGGTACAAAATCAATGATTGCGGTCAGGAGGTGGAGTATTTC
B-Hcp1-A95C	GAAATACTCCACCTCCTGACCGCAATCATTGATTTTGTACCAC
A-Hcp1-G96C	GTACAAAATCAATGATGCCTGTCAGGAGGTGGAGTATTTC
B-Hcp1-G96C	GAAATACTCCACCTCCTGACAGGCATCATTGATTTTGTAC
A-Hcp1-C38S	GTGGTGGCTCAGGAGCACAGCCTCTACATCCCGACGG
B-Hcp1-C38S	CCGTCGGGATGTAGAGGCTGTGCTCCTGAGCCACCAC
A-Hcp1-S158W	CATTCATTCCGACGCATGGTGGGAGCGTACCACCGCGTAA
B-Hcp1-S158W	TTACGCGGTGGTACGCTCACCCCATGCGTCGGAATGAATG

[a] Sequences corresponding to the downstream and upstream regions of the gene to be deleted underlined.
[b] Sequence annealing on the target plasmid underlined.
[c] FLAG or VSV-G epitope coding sequence in **Bold**
[d] 3×Ala-3×Gly linker in *italics*.
[e] Mismatch codon underlined.