Appendix

Appendix Table S1

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

Strains

Strains	Description and genotype	Source
E. coli K-12		
DH5α	F-, Δ(*argF-lac*)U169, *phoA*, *supE44*, Δ(*lacZ*)M15, *relA*, *endA*, *thi*, *hsdR*	New England Biolabs
W3110	F-, lambda- IN(*rrnD-rrnE*)1 *rph*-1	Laboratory collection
BTH101	F-, *cya-99, araD139, galE15, galK16, rpsL1 (Str r), hsdR2, mcrA1, mcrB1.*	Karimova *et al.*, 2005
BL21(DE3)	F-, *fhuA2*, *lon*, *ompT*, *gal (λ int::(lacI::PlacUV5::T7 gene1)*, *dcm*, Δ*hsdS*	New England Biolabs
MC1061	F-, *araD139 Δ(ara-leu)*, *galE15*, *galK16*, Δ*(lac)X74*, *rpsL* (Str^R) *hsdR2 mcrAB1*	New England Biolabs
Enteroaggregative *E. coli*		
17-2	WT enteroaggregative *Escherichia coli*	Arlette Darfeuille-Michaud
17-2Δ*tssM*	17-2 deleted of the *tssM* gene of the *sci1* T6SS gene cluster	Aschtgen *et al.*, 2010
17-2Δ*mltA*	17-2 deleted of the *mltA* gene	This study
17-2Δ*mltB*	17-2 deleted of the *mltB* gene	This study
17-2Δ*mltC*	17-2 deleted of the *mltC* gene	This study
17-2Δ*mltD*	17-2 deleted of the *mltD* gene	This study
17-2Δ*mltE*	17-2 deleted of the *mltE* gene	This study
17-2Δ*slt70*	17-2 deleted of the *slt70* gene	This study
17-2Δ*2762*	17-2 deleted of the *EC042_2762* gene	This study
17-2Δ*etgA*	17-2 deleted of the *etgA* gene	This study
17-2 sfGFP-TssM	sfGFP inserted after the start codon of the *tssM* gene in 17-2	Durand *et al.*, 2015
17-2Δ*mltE* sfGFP-TssM	17-2 sfGFP-TssM deleted of the *mltE* gene	This study

Plasmids

Vectors	Description	Source
Vectors for chromosomal insertions		
pKD4	One-step gene inactivation vector, Kan^R	Datsenko & Wanner, 2000
pKOBEG	Recombination vector, phage *λ recγβα* operon under the control of the pBAD promoter, Cm^R	Chaveroche *et al.*, 2000
Expression vectors		
pUA66-*rrnb*	P_{rrnB} *::gfpmut2* transcriptional fusion in pUA66, Kan^R	Zaslaver *et al.*, 2006
pASK-IBA37(+)	cloning vector, P*tet*, f1 origin, Amp^R	IBA Technology
pASK-IBA37-$_{FLAG}$TssM	*sci-1 tssM* carrying N-terminal FLAG tag cloned into pASK-IBA37(+)	Aschtgen *et al.*, 2010
pASK-IBA4	cloning vector, P*tet*, OmpA signal sequence, f1 origin, Amp^R	IBA Technology
pASK-IBA4-TssMp	*sci-1 tssM* periplasmic domain (aa 386-1129), cloned into pASK-IBA4, N-terminal FLAG epitope	Felisberto-Rodrigues *et al.*, 2011
pASK-IBA4-$TssM_{386-973}$	*sci-1 tssM* periplasmic fragment (aa 386-973), cloned into pASK-IBA4, N-terminal FLAG epitope	Felisberto-Rodrigues *et al.*, 2011
pASK-IBA4-$TssM_{972-1129}$	*sci-1 tssM* periplasmic fragment (aa 972-1129), cloned into pASK-IBA4, N-terminal FLAG epitope	Felisberto-Rodrigues *et al.*, 2011
pMS600	cloning vector, pOK12 derivative, P*lac*, P15A origin, Kan^R	Aschtgen *et al.*, 2008
pMS-Hcp_{HA}	*sci-1 hcp* gene cloned into pMS600, C-terminal HA epitope	Aschtgen *et al.*, 2010
pMS-$TssJ_{HA}$	*sci-1 tssJ* gene cloned into pMS600, C-terminal HA epitope	Aschtgen *et al.*, 2008
pMS-$TssL_{HA}$	*sci-1 tssL* gene cloned into pMS600, C-terminal HA epitope	Aschtgen *et al.*, 2012
pUC12	cloning vector, P*lac*, ColE1 origin, Amp^R	Norrander *et al.*, 1983
pUC-Hcp_{FLAG}	*sci-1 hcp* gene cloned into pUC12, C-terminal FLAG epitope	Aschtgen *et al.*, 2008
pBAD33	cloning vector, *Para*, *araC,* p15A origin, Cm^R	Guzman *et al.*, 1995
pBAD33-$MltE_{VSV-G}$	*mltE* gene cloned into pBAD33, C-terminal VSV-G epitope	This study
pBAD33-$MltE^{E64Q}$	*mltE* Glu64-to-Gln mutation introduced in pBAD33-$MltE_{VSV-G}$, C-terminal VSV-G epitope	This study
pETG20A	Gateway® destination cloning vector, *PT7*, N-terminal 6×His tag, TEV clivage sequence, ColE1 origin, Amp^R	Arie Gerlof
pETG20A-TssMp	*sci-1 tssM* periplasmic domain (aa 386-1129), cloned into pETG20A	Felisberto-Rodrigues *et al.*, 2011
pBADnLIC	cloning vector, *Para*, *araC*, N-terminal 10×His tag, TEV clivage sequence, P15A origin, Amp^R	Geertsma & Poolman, 2007
pBADnLIC-sMltE	*mltE* gene lacking its signal sequence cloned into pBADnLIC	Fibriansah *et al.*, 2012
pBADnLIC-$MltE^{E64Q}$	*mltE* Glu64-to-Gln mutation introduced in pBADnLIC-MltE	Fibriansah *et al.*, 2012
Bacterial two-hybrid vectors		
pT18-FLAG	bacterial two hybrid vector, ColE1 origin, P*lac*, T18 fragment of *Bordetella pertussis* CyaA, Amp^R	Battesti & Bouveret, 2008

pT18-MltA	*mltA* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-MltB	*mltB* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-MltC	*mltC* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-MltD	*mltD* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-MltE	*mltE* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-MltE^{E64Q}	*mltE* Glu64-to-Gln mutation introduced in pT18-MltE	This study
pT18-Slt70	*slt70* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-2762	*EC042_2762* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-EtgA	*etgA* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	This study
pT18-Pal	*pal* gene lacking its signal sequence cloned downstream T18 in pT18-FLAG	Battesti & Bouveret, 2008
pT25-FLAG	bacterial two hybrid vector, P15A origin, P*lac*, T25 fragment of *Bordetella pertussis* CyaA, Kan^R	Battesti & Bouveret, 2008
pT25-VgrG	*sci-1 vgrG* gene cloned downstream T25 in pT25-FLAG	Zoued *et al.*, 2013
pT25-TssJ$_{sol}$	*sci-1 tssJ* gene lacking its signal sequence cloned downstream T25 in pT25-FLAG	Zoued *et al.*, 2013
pT25-TssMp	*sci-1 tssM* periplasmic domain (amino-acids 386-1129) cloned downstream T25 in pT25-FLAG	Zoued *et al.*, 2013
pT25-TagLp	*sci-1 tagL* periplasmic domain (amino-acids 352-576) cloned downstream T25 in pT25-FLAG	This study
pTolB-T25	*tolB* gene cloned upstream T25 in pT25-FLAG	Battesti & Bouveret, 2008

Oligonucleotides

Name	Destination	Sequence (5' to 3')
For chromosomal mutant strain construction [a]		
5-Del-mltA-DW	*mltA* gene deletion	CGGTTTGTTATCTTCGTTGCGCCTTATTTTTTAACCTGAAGAAGAGAACATGTGTAGGCTGGAGCTGCTTCG
3-Del-mltA-DW	*mltA* gene deletion	TTACCCCTCACCCTGTCATATCCGTAAAAACGGCATACAGAATATCACACATATGAATATCCTCCTTAGTTC
5-Del-mltB-DW	*mltB* gene deletion	TGATGCTTTACCATACTTGCCCCTGGTTGAATCTGTTAAATGGACCCCTCTGTGTAGGCTGGAGCTGCTTCG
3-Del-mltB-DW	*mltB* gene deletion	AAAAGCTGATTAGCCAGAGGGAAGCTCACGCTCCCCTCTTGCAAATAGCATATGAATATCCTCCTTAGTTC
5-Del-mltC-DW	*mltC* gene deletion	GCACGCCTCCGGCAACTTGCATAAAAACAAACACAACACGCACCCGGATGTGTAGGCTGGAGCTGCTTCG
3-Del-mltC-DW	*mltC* gene deletion	TGTGGATAACATTTTTGCCCCTGAGCATCGTCAGGGGCGGTTAATGGAACATATGAATATCCTCCTTAGTTC
5-Del-mltD-DW	*mltD* gene deletion	TCCGTTCGCCGTTATGATCGGTCGTCTTTTAAGCAACTATTGACACACACTGTGTAGGCTGGAGCTGCTTCG
3-Del-mltD-DW	*mltD* gene deletion	AAATAAAAAAGGCACCGGGGGAATCGGTGCCTTTTTATTATCTGGTTTGCATATGAATATCCTCCTTAGTTC
5-Del-mltE-DW	*mltE* gene deletion	TGTGCCGTTGTCACCTCAACGGCGATTCCAGGCTATAAGGATAGAAGAATGTGTAGGCTGGAGCTGCTTCG
3-Del-mltE-DW	*mltE* gene deletion	CTCTCGAGCGGGAAGCCCGGGAGAAAGCGGACAAAGTGCGCGACTGATCATATGAATATCCTCCTTAGTTC
5-Del-slt70-DW	*slt70* gene deletion	TTACGCGGCATGACGCTGCATTGATGTATTTACACTTAGAGGATGCGCTTTGTGTAGGCTGGAGCTGCTTCG

3-Del-slt70-DW	*slt70* gene deletion	CCGGTTGTACTCGCTAAAGAGTACGATAGCATATCATAAACGTGCGGACATATGAATATCCTCCTTAGTTC
5-Del-2762-DW	*EC042_2762* gene deletion	TTATTACGTTTTTTCAAGCTGGGACGCGCACGACACAGAGAATTAACTAATGTGTAGGCTGGAGCT GCTTCG
3-Del-2762-DW	*EC042_2762* gene deletion	ATGCGCCGCCAGGAAATTAAAGCGCAGAAAAAAGCGCGATCCTCGACGGACATATGAATATCCTC CTTAGTTC
5-Del-etgA-DW	*etgA* gene deletion	GATTATATTAGTATCTCGTTCCTTTCCTTCAATCCTACACATAAAAATATTGTGTAGGCTGGAGCTGCTTCG
3-Del-etgA-DW	*etgA* gene deletion	AGCTTTACGTATGGGTGTTTGCACTATATAAAAAAAAGAGGCTTTAGGCCATATGAATATCCTCCTTAGTTC

For plasmid construction [b,c]

5-pBAD-MltE$_{VSV-G}$	insertion of *mltE* into pBAD33	CTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGTATTACACCATGAAATT AAGATGGTTTGCCTTTTTGATTGTG
3- pBAD-MltE$_{VSV-G}$	insertion of *mltE* into pBAD33	GGTCGACTCTAGAGGATCCCCGGGTACCTTA*TTTTCCTAATCTATTCATTTCAATATCTGTATA*C ATCGCGTCCAGTGCCTGC
T18N-5-MltA	insertion of signal sequence-less *mltA* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGTCTTCCAAACCAAC CGATCGCG
T25T18N-3-MltA	insertion of signal sequence-less *mltA* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTAGCCGCTA AAGACGTTACCTGCG
T18N-5-MltB	insertion of signal sequence-less *mltB* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGAGCAGCAAGCCAA AACCTACTGAG
T25T18N-3-MltB	insertion of signal sequence-less *mltB* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACTGCACG CGCGCCAG
T18N-5-MltC	insertion of signal sequence-less *mltC* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGTCGACGACCAAAA AAGGCGATACC
T25T18N-3-MltC	insertion of signal sequence-less *mltC* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTATCGGCGG CGGTAGGATTTTTG
T18N-5-MltD	insertion of signal sequence-less *mltD* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGCAGAGTACCGGCA ACGTTCAAC
T25T18N-3-MltD	insertion of signal sequence-less *mltD* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTAGGAATCT GGCATGTTGTTGTTTTTCACAAAC
T18N-5-MltE	insertion of signal sequence-less *mltE* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGTCATCAAAGCATGA CTACACGAACCC
T25T18N-3-MltE	insertion of signal sequence-less *mltE* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACATCGCGT CCAGTGCCTG
T18N-5-Slt70	insertion of signal sequence-less *slt70* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGGCGCGAGCCGACT CACTG
T25T18N-3-Slt70	insertion of signal sequence-less *slt70* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTAGTAACGA CGTCCCCATTCCGTG

T18N-5-2762	insertion of signal sequence-less *EC042_2762* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGGCTCTCTGG CCATCCATTCC
T25T18N-3-2762	insertion of signal sequence-less *EC042_2762* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTAAT TTTGTTTCTCTTCACTCCCTTTCCTGG
T18N-5-EtgA	insertion of signal sequence-less *etgA* into pT18-FLAG	CGCCACTGCAGGGATTATAAAGATGACGATGACAAGGCCAGTAGCGCTTG CTTTAATGAAGC
T25T18N-3-EtgA	insertion of signal sequence-less *etgA* into pT18-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTATTTTGCTA AAGCCTTACGCTTGTCTATTTC
T25N-5-TagLp	insertion of $tagL_{352\text{-}576}$ fragment into pT25-FLAG	GGCGGGCTGCAGATTATAAAGATGACGATGACAAGCGGCTGGTTCGCAGC GTG
T25T18N-3-TagLp	insertion of $tagL_{352\text{-}576}$ fragment into pT25-FLAG	CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAGTTACTCCGTT ATGTTTTCTGATGCGCC
For site-directed mutagenesis [d]		
A-MltE-E64Q	Glu64-to-Gln mutation in *mltE*	GGCGATTATCGCTATC**CAA**TCGGGTGGTAATCC
B-MltE-E64Q	Glu64-to-Gln mutation in *mltE*	GGATTACCACCCGA**TTG**GATAGCGATAATCGCC

[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 *italicized*.
[d] Mutagenesized codon in **Bold.**