The Hcp-like protein HilE inhibits homodimerization and DNA binding of the virulence-associated transcriptional regulator HilD in *Salmonella*

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Running title: *HilE controls dimerization and DNA binding of HilD*

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Supporting information

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
Strains		
S. Typhimurium strains		
SL1344	Wild type; *xyl hisG rpsL;* Sm^R	(1)
14028s	Wild type	ATCC
JPTM5	Δ*hilD::kan*	(2)
E. coli K-12 strains		
DH5α	Laboratory strain	Invitrogen
SU101	Reporter strain of the LexA-based genetic system for homodimerization assays; Kan^R	(3)
SU202	Reporter strain of the LexA-based genetic system for heterodimerization assays; Kan^R	(3)
BL21/DE3	Strain for expression of recombinant protein	Invitrogen
MC4100	Cloning strain	(4)
Plasmids		
pSR658	Vector expressing $LexA_{DBDwt}$ for homodimerization assays; Tc^R	(5)
pSR658-HilD1	pSR658 derivative expressing $LexA_{DBDwt}$-$HilD_{1-309}$; Tc^R	This study
pSR658-HilD2	pSR658 derivative expressing $LexA_{DBDwt}$-$HilD_{1-130}$; Tc^R	This study
pSR658-HilD3	pSR658 derivative expressing $LexA_{DBDwt}$-$HilD_{130-309}$; Tc^R	This study
pSR658-HilD4	pSR658 derivative expressing the $LexA_{DBDwt}$-$HilD_{1-220}$; Tc^R	This study
pSR658-HilD5	pSR658 derivative expressing the $LexA_{DBDwt}$-$HilD_{221-309}$; Tc^R	This study
pSR658-HilD6	pSR658 derivative expressing the $LexA_{DBDwt}$-LZ-$HilD_{221-309}$; Tc^R	This study
pSR658-HNS	pSR658 derivative expressing $LexA_{DBDwt}$-H-NS; Tc^R	(6)
pSR659	Vector expressing $LexA_{DBDmut}$ for heterodimerization assays; Ap^R	(5)
pSR659-HilE1	pSR659 derivative expressing the $LexA_{DBDmut}$-HilE fusion; Ap^R	This study
pMAL-c2x	Expressing vector for constructing Maltose binding protein (MBP) fusions, *lac* promoter; Ap^R	New England Biolabs
pMAL-HilD1	pMAL-c2x derivative expressing MBP-HilD fusion protein; Ap^R	(2)
pMPM-A6Ω	Cloning vector p15A derivative low-copy-number, *ara* promoter; Ap^R	(7)

pA6-HilE1	pMPM-A6Ω derivative expressing HilE; Ap^R	This study
pMPM-K6Ω	Cloning vector p15A derivative low-copy-number, *ara* promoter; Kan^R	(7)
pK6-HilE1	pMPM-K6Ω derivative expressing HilE; Kan^R	This study
pUT18C-zip	Vector expressing 35 amino acids of the leucine zipper motif from GCN4 fused in frame with the T18 fragment of CyA; Ap^R	Euromedex
pET32b(+)	Expressing vector for constructing Thioredoxin (Trx) fusions, T7 promoter; Ap^R	Novagen
pET32-HilE	pET32b(+) derivative expressing Trx-HilE; Ap^R	This study
pBAD-HilD1	pBADMycHis derivative expressing HilD-MycHis; Ap^R	(6)
philA-cat1	pKK232-8 derivative expressing a *hilA-cat* transcriptional fusion from nucleotides - 410 to + 446	(2)

Ap^R, ampicillin resistance; Sm^R streptomycin resistance; Kan^R, kanamycin resistance; Tc^R, tetracycline resistance.

Table S2. Primers used in this study

Primer	Sequence (5'-3')	Target gene	RE
HilD-SacI	GAT GAG CTC GAA AAT GTA ACC TTT GTA AGT AAT AG	*hilD*	SacI
HilDexR-PstI	TCC CTG CAG AAC AAT GAT ATT GAA TAG C	*hilD*	PstI
HilD130-5'	CCC ACA GAG CTC GCG CAG AAG ATC TTC TAT ACG	*hilD*	SacI
HilD-130	ATA GAA CTG CAG TTA CGC TTT CTC TGT GGG TAC CG	*hilD*	PstI
HilD-220	AAG CTT CTG CAG TTA ACT GGG TGA CGA AGA TAT AAT G	*hilD*	PstI
HilD-221	TCG TCA GAG CTC AGA CAG TGG AAG CTT ACG GAT G	*hilD*	SacI
LZ-F	GTA CTC GAG CAG CGT ATG AAA CAG CTG GAA G	*gcn4**	XhoI
LZ-HilD221F	GAA AAA ACT GGT GGG TGA ACG TAG ACA GTG GAA GCT TAC GGA TG	*gcn4**	
HilD221LZR	CAT CCG TAA GCT TCC ACT GTC TAC GTT CAC CCA CCA GTT TTT TC	*hilD*	
HilE-SacI	GGG GAG CTC GAC GCC ATC TAT TTA AAA CTG G	*hilE*	SacI
HilE- HindIII-3'	TCC GCA AGC TTG TTT TGT CC	*hilE*	HindIII
HilE-NcoI-2	GAG GGG CCA TGG ACG CCA TCT ATT TAA AAC TGG	*hilE*	NcoI
HilE-His6	CCC AAG CTT TCC TCA ATG ATG ATG ATG ATG ATG TCG CCA CAG CGC CTG TCG G	*hilE*	HindIII
HilE-PUT-BamHI	ATA ACG GAT CCG AGG GCC ACG CGT TAT CGC	*hilE*	BamHI
HilCRR-F	GGA ATG TAA TTA TTG GCT ATA ATA ATA AAA AAA TCG GAT TTA AAT CAT CT	*hilC*	
HilCRR-R	GAG ATG ATT TAA ATC CGA TTT TTT TAT TAT TAT AGC CAA TAA TTA CAT TC	*hilC*	

RE, restriction enzyme for which a site was generated in the primer. Underlined letters indicate the respective restriction-enzyme site in the primer. * *gcn4* carried by pUT18C-zip (Table 1).

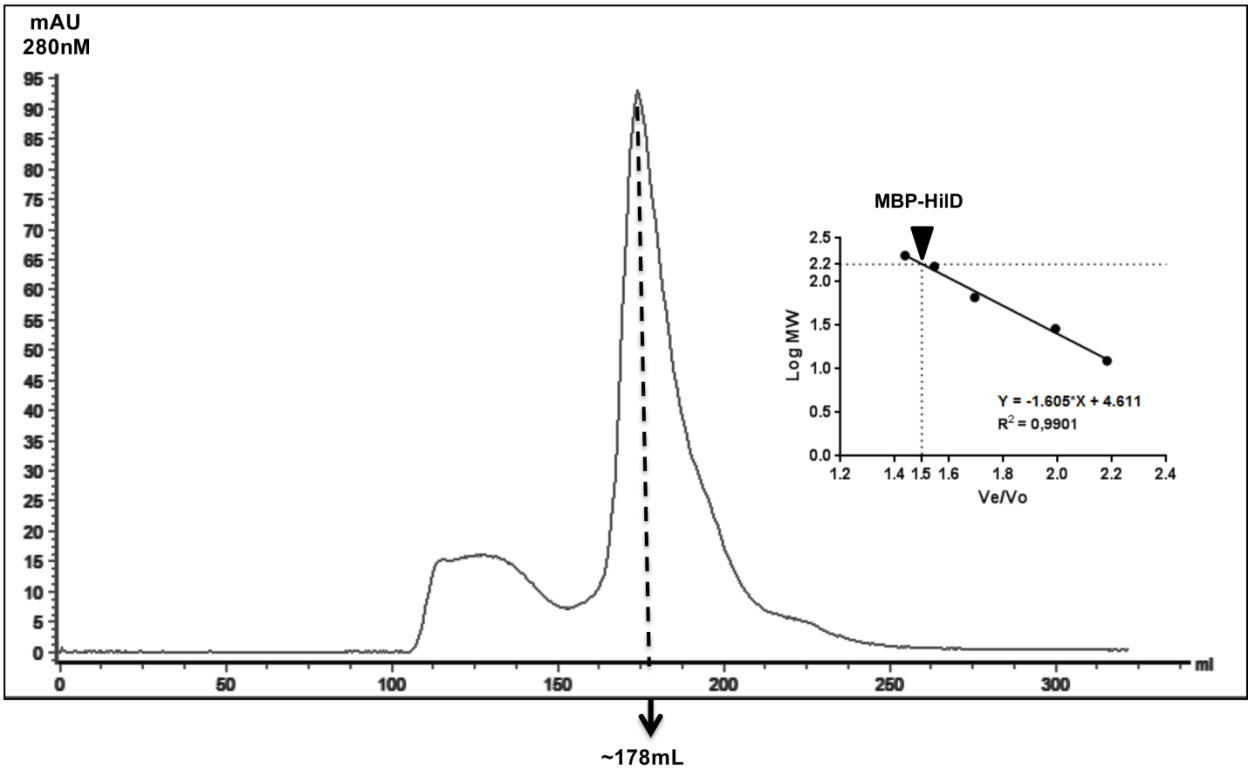

Figure S1. Gel filtration chromatography of MBP-HilD. Chromatogram showing the elution profile of MBP-HilD, fractionated on a Superdex 200 column (HiLoad^{TM} 16/60), in a buffer containing 200 mM Tris–HCl pH 8.0 and 150 mM NaCl. On the right, a five-point calibration curve was performed using the Gel Filtration Molecular Weight Markers Kit (Sigma-Aldrich). The arrow indicates the relative molecular mass of MBP-HilD calculated by comparison with the five-point calibration curve.

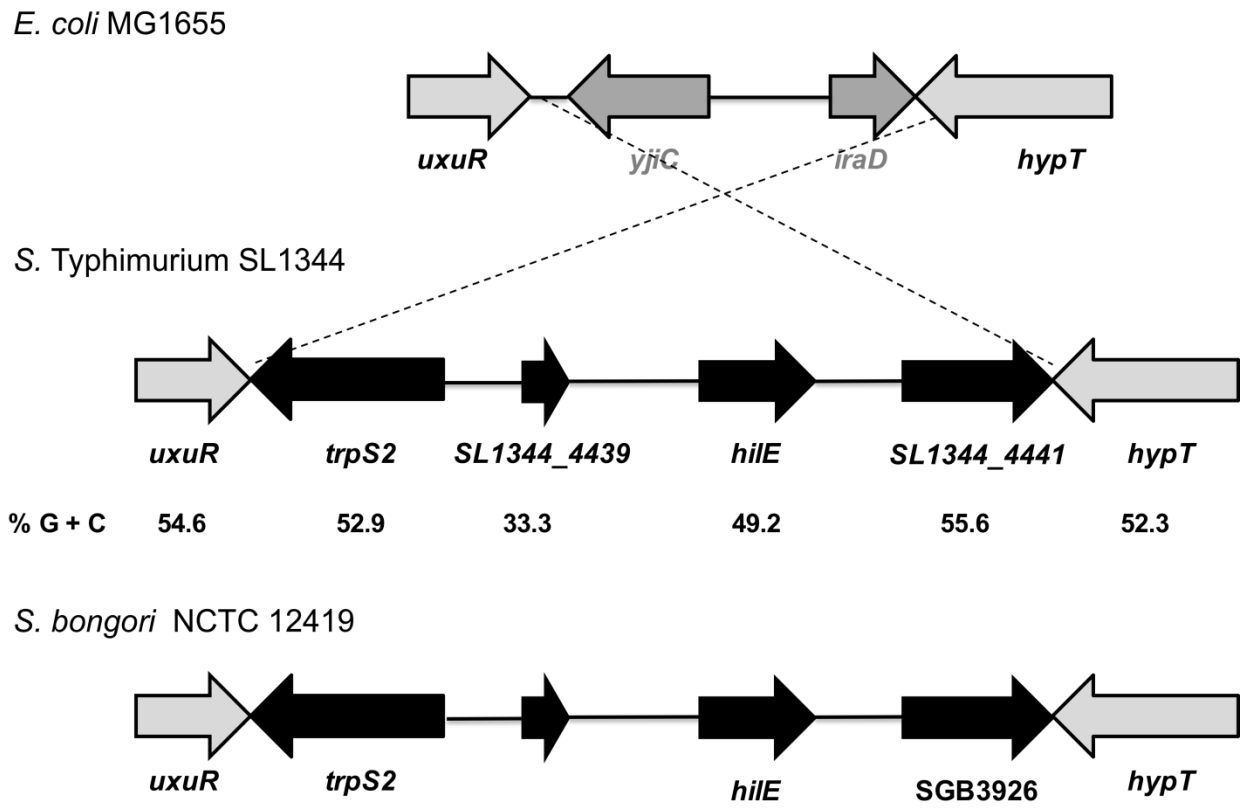

Figure S2. The *hilE* gene is located in a genomic island of *S.* Typhimurium and *S. bongori* that is absent in *E. coli* K-12. Schematic view of the DNA region between the *uxuR* and *hypT* ancestral genes in *E. coli* K-12 MG1655, *S.* Typhimurium SL1344 and *S. bongori* NCTC 12419. The *hilE* gene is present in *S.* Typhimurium and *S.* bongori, but not in *E. coli* K-12. The *trpS2*, *SL1344_4438* and *SL1344_4431* genes encode for a tryptophanyl-tRNA systethase, a hypothetical protein and a putative aspartate-racemase, respectively. The G + C content for each of the *S.* Typhimurium SL1344 genes is shown.

Supplementary references

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