Differential regulation of the *hmsCDE* operon in *Yersinia pestis* and *Yersinia pseudotuberculosis* by the Rcs phosphorelay system

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Supplementary Tables

Supplementary Table S1: Strains and plasmids used in this study

Strain or plasmid	Genotype and/ or description	Reference or source
Y. pestis		
KIM6+	wild type (pCD1-), nonfunctional *rcsA*	1
CDY326	Δ*rcsB*::*Km*	2
SY591	Δ*hmsCDE*	This study
SY1567	Δ*rcsD*-N-terminal, *hmsD*-Myc_2	This study
SY1568	Δ*rcsB*::*Km*, *hmsD*-Myc_2	This study
SY1570	*hmsD*-Myc_2	This study
SY1615	*hmsD*-Myc_2, RcsAXX[a]	This study
SY1726	functional *rcsA*, *hmsD*-Myc_2	This study
SY1783	functional *rcsA*, *hmsD*-Myc_2, RcsAXX	This study
SY1784	functional *rcsA*, *hmsD*-Myc_2, RcsABX[b]	This study
SY1785	*hmsD*-Myc_2, RcsXAB[c]	This study
SY1786	*hmsD*-Myc_2, RcsABX	This study
SY1788	Δ*rcsB*::*Km*, *hmsD*-Myc_2, RcsAXX	This study
SY1790	Δ*rcsB*::*Km*, *hmsD*-Myc_2, RcsABX	This study
SY1856	functional *rcsA*, *hmsD*-Myc_2, RcsXXX[d]	This study
SY1860	*hmsD*-Myc_2, RcsXXX	This study
SY1861	Δ*rcsB*::*Km*, *hmsD*-Myc_2, RcsXXX	This study
SY1910	Δ*rcsB*::*Km*, *hmsD*-Myc_2, RcsXAB	This study
SY1911	functional *rcsA*, *hmsD*-Myc_2, RcsXAB	This study
Y. pseudotu-berculosis		
IP32953	wild type; serogroup O1	3
CDY331	nonfunctional *rcsA*	2

SY1798	*hmsD*-Myc_2	This study
SY1799	nonfunctional *rcsA*, *hmsD*-Myc_2	This study
SY1937	Δ*rcsB*::*Km*, *hmsD*-Myc_2	This study
SY1939	nonfunctional *rcsA*, *hmsD*-Myc_2	This study
SY1966	*hmsD*-Myc_2, RcsAXX	This study
SY1967	nonfunctional *rcsA*, *hmsD*-Myc_2, RcsAXX	This study
SY1968	Δ*rcsB*::*Km*, *hmsD*-Myc_2, RcsAXX	This study
SY1969	Δ*rcsB*::*Km*, nonfunctional *rcsA*, *hmsD*-Myc_2, RcsAXX	This study

Plasmids

pBAD/ Myc-His	expression vector, araBAD promoter, Amp^R	Invitrogen
pUC19	cloning vector for PCR products, Amp^R	4
pGD926	*lacZ* translational fusion vector, Tet^R	5
pVTRA	low level expression plasmid, IPTG induced, Cat^R	6
pCBD179	functional *rcsA* in pUC18	2
pCBD209	*rcsB* in pBAD/ Myc-His	7
pYC287	*hmsC* 5' UTR::*lacZ* fusion in pGD926	This study
pYC300	*hmsC* 5' UTR (RcsAXX)::*lacZ* fusion in pGD926	This study
pYC301	*hmsC* 5' UTR (-10 box*[e])::*lacZ* fusion in pGD926	This study
pYC332	*rcsB* in pUC19	This study
pYC484	*hmsC* 5' UTR (RcsXXX)::*lacZ* fusion in pGD926	This study
pYC485	*hmsC* 5' UTR (RcsXAB)::*lacZ* fusion in pGD926	This study
pYC486	*hmsC* 5' UTR (RcsABX)::*lacZ* fusion in pGD926	This study
pYC487	*hmsC*::*lacZ* fusion in pGD926	This study
pYC513	*hmsCDE* in pVTRA	This study
PYC575	*rcsB*(D56Q) in pUC19	This study
PYC576	*rcsB*(D56E) in pUC19	This study
PYC593	*hmsT* 5' UTR::*lacZ* fusion in pGD926	This study
PYC597	*lcrQ* 5' UTR::*lacZ* fusion in pGD926	This study

[a]RcsAXX: RcsAAB mutation showed as Figure 3: TAAGATT**TCTGTTTCGCTTTT** (the mutated base pairs are bold, the same below).

[b]RcsABX: RcsAAB mutation showed as Figure 3: TAAGATAAATCTCA**CGCTTTT**

[c]RcsXAB: RcsAAB mutation showed as Figure 3: **CTTTCTG**TAAGATAAATCTCA

[d]RcsXXX: RcsAAB mutation showed as Figure 3: **CTTTCTGTCTGTTTCGCTTTT**

[e]-10 box*: -10 box mutation of *hmsC* promoter.

Supplementary Table S2: Oligonucleotides used in this study

Construction of *rcsB* in pUC19 (pYC332)

cgggatccatttaagcagcgcggttat

ggaattctactacagtgaccaagtacggcg

Construction of *lacZ* deletion

atgacgtcacaggaaaaggtaccactccaggtgcaactgagtcttgtgtaggctggagctgcttcg

ttacaccttgtattgccaacagatttggtactgataggtttcacgcatatgaatatcctccttag

Construction of *hmsC* 5' UTR::*lacZ* fusion in pGD926 (pYC287)

cccaagcttgtccagcccagctccg

cgggatccgcgcaagtagcggtagtc

Construction of *hmsC*::*lacZ* fusion in pGD926 (pYC487)

cccaagcttatgactaccgctacttgcg

cgggatcctgatttttttcggcttcat

Construction of *hmsT* 5' UTR::*lacZ* fusion in pGD926 (pYC593)

caagcttcacggctgagcaacccgg

cgcggatccatattcaatttactctgca

Construction of *lcrQ* 5' UTR::*lacZ* fusion in pGD926 (pYC597)

ccaagcttgcgtttggatcaaggggg

cgcggatcctgaagagtattgattttcatcg

Determination of *hmsC* transcription start site

attctggcagcagcatcgctacgttcacgc

gcattgatattactggcggccaataatggggttggtac

agccaccaataatgaaagtaa

cgacgttattataagatttatatga

PCR amplification of *hmsC* promoter for EMSA

tacctgacaaaaacccggaaa

tgatgtaataatagtcatatcatcgt

PCR amplification of *hmsT* promoter for EMSA

tcatgatgacaggctgaaaca

tccgacatcacgacaaataaa

PCR amplification of *lcrQ* promoter for EMSA

cccaaaaataatttttattgtgattt

tataattgtctctacgatattctaagttatttatt

RcsB (D56Q) mutagenesis

aattactcaactctctatgccaggggataagtatggtgatggcatcac
agagttgagtaattagcacgttggcatcaagtttggacaaattgttaataagc

RcsB (D56E) mutagenesis

aattactgaactctctatgccaggggataagtatggtgatggcatcac
agagttcagtaattagcacgttggcatcaagtttggacaaattgttaataagc

RcsAXX box mutagenesis

tctgtttcgcttttaatttacgatgatatgactattattacatcatataaatcttataataacg
aaaagcgaaacagaaatcttatttatgttattgaaatagaaatataaaaaacccga

RcsXXX box mutagenesis

ctttctgtctgtttcgcttttaatttacgatgatatgactattattacatcatataaatcttataataacg
aaaagcgaaacagacagaaagtttatgttattgaaatagaaatataaaaaacccgactgtt

RcsXAB box mutagenesis

ctttctgtaagataaatctcaaatttacgatgatatgactattattacatcatataaatcttataataacg
tgagatttatcttacagaaagtttatgttattgaaatagaaatataaaaaacccgactgtt

RcsABX box mutagenesis

aatctcacgcttttaatttacgatgatatgactattattacatcatataaatcttataataacg
aaaagcgtgagattaatcttatttatgttattgaaatagaaatataaaaaacccga

-10 box mutagenesis

ttaggtacgacgttagatctagatttatatgatgtaataatagtcatatcatcgtaaattaagatctttctgtc
agatctaacgtcgtacctaacgccgattcaaccaccacattta

***hmsD* qRT-PCR**

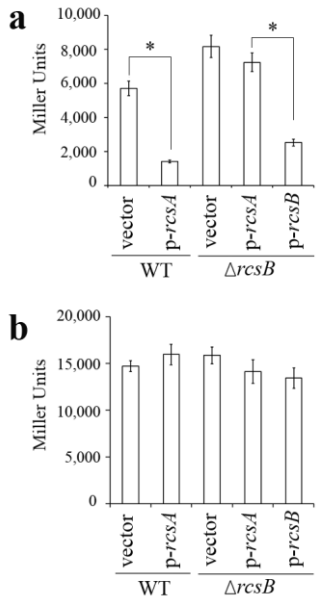
acctgcatttgatcgcccgcg36-TAMSp
gccttacgggtttatgttgatcac
ggcctcggtggtataactgatg

***crr* qRT-PCR**

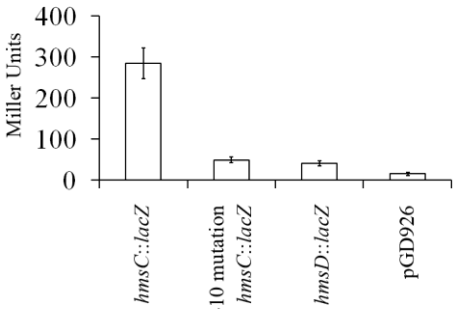
ctcctgttgacggcaccatcggt36-TAMSp
gccctctggcaataaaatgg
agcatggttggtctcgaaaatt

All sequences are in 5´-3´orientation.

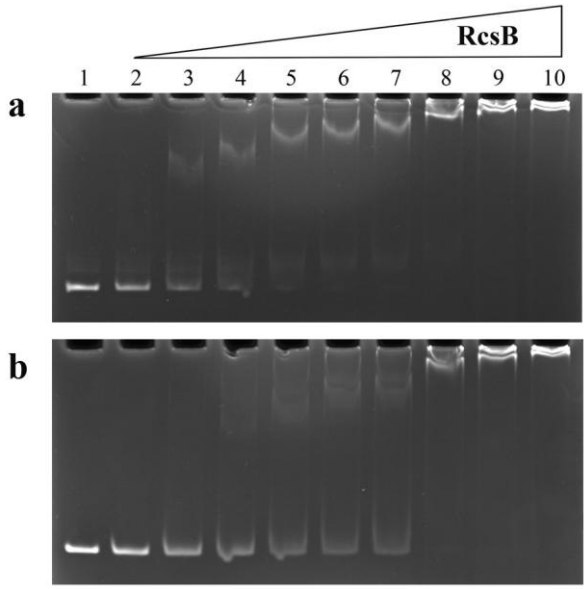
Supplementary Figures



Supplementary Figure S1: β-galactosidase activities of *hmsT*::*lacZ* and *lcrQ*::*lacZ* reporter. β-galactosidase activities of *hmsT*::*lacZ* (a) and *lcrQ*::*lacZ* (b) reporter in *Y. pestis*. *Y. pestis* KIM6+ (WT) transformed with empty vector (vector) and functional RcsA (p-*rcsA*), RcsB deletion mutant transformed with empty vector (vector), wild-type RcsA (p-*rcsA*) and wild-type RcsB (p-*rcsB*). The mean and standard deviation of three independent experiments with three replicates are indicated. **P*<0.01.

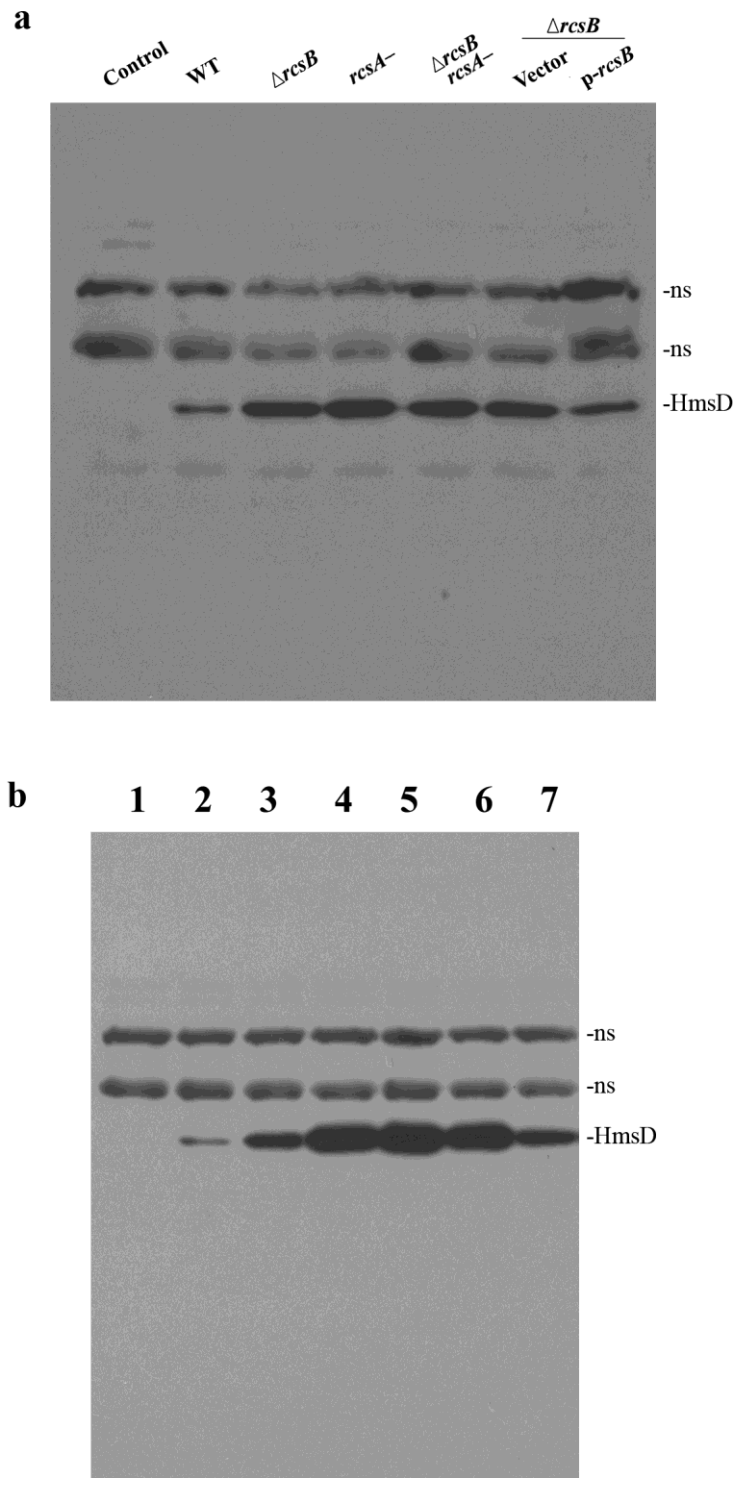


Supplementary Figure S2: β-galactosidase activities of *hmsC*::*lacZ* and *hmsD*::*lacZ* reporters. β-galactosidase activities of *hmsC*::*lacZ* and *hmsD*::*lacZ* reporters in *Y. pestis*. KIM6+ (WT) transformed with *hmsC*::*lacZ*, *hmsC*::*lacZ* with mutated putative -10 box, *hmsD*::*lacZ* and pGD926 plasmids. The mean and standard deviation of three independent experiments with three replicates are indicated. **P*<0.05, ***P*<0.01.



Supplementary Figure S3: EMSA results of RcsB with *hmsT* and *lcrQ* promoter.

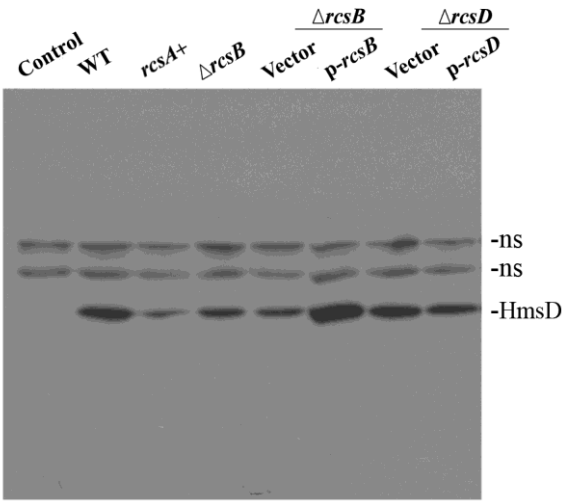
Electrophoretic mobility shift assays (EMSA) of *hmsT* (a) and *lcrQ* (b) promoter DNA incubated with increasing concentrations of RcsB. Probes were tested with identical protein combinations. Lane 1, probe alone; lanes 2-10, 100 ng probe with 400, 800, 1200, 1600, 2000, 2400, 4000, 6000 or 8000 ng of RcsB in the 16 μL reaction.



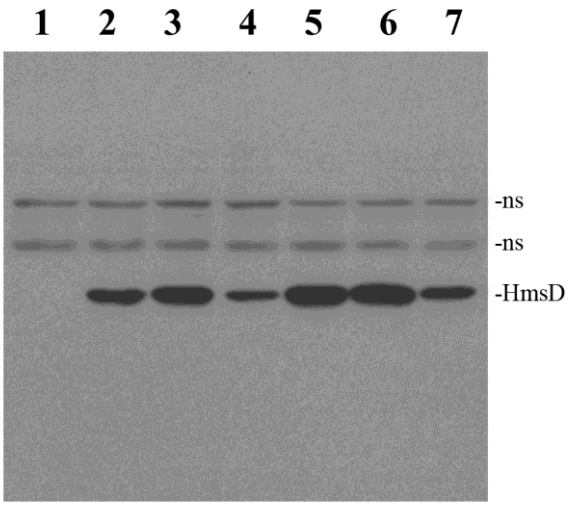
Supplementary Figure S4: Role of the RcsAAB box on regulation of expression of HmsD by Rcs in *Y. pseudotuberculosis*. Western blots of total protein-matched lysates prepared from cells with (a) RcsAAB box and (b) RcsAXX box were analyzed by anti-Myc antibody. Strain designations (Supplementary Table S1) are: (a) Control, IP32953; WT, *hmsD*-Myc_2; Δ*rcsB*,

Δ*rcsB hmsD*-Myc$_2$; *rcsA*-, nonfunctional *rcsA hmsD*-Myc$_2$; Δ*rcsB rcsA*-, nonfunctional *rcsA* Δ*rcsB hmsD*-Myc$_2$; Δ*rcsB* Vector, Δ*rcsB hmsD*-Myc$_2$/ pUC19; Δ*rcsB* p-*rcsB*, Δ*rcsB hmsD*-Myc$_2$/ pYC332.

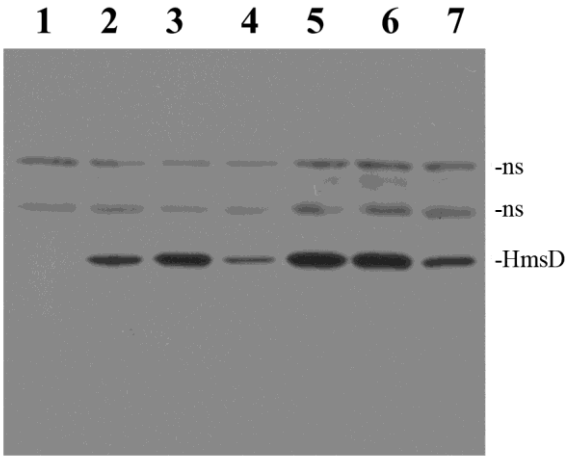
(b) 1, IP32953; 2, *hmsD*-Myc$_2$; 3, RcsAXX mutation, *hmsD*-Myc$_2$; 4, nonfunctional *rcsA*, RcsAXX mutation, *hmsD*-Myc$_2$; 5, Δ*rcsB*, RcsAXX mutation, *hmsD*-Myc$_2$; 6, Δ*rcsB*, RcsAXX mutation, *hmsD*-Myc$_2$/ pUC19; 7, Δ*rcsB*, RcsAXX mutation, *hmsD*-Myc$_2$/ pYC332. pYC332: p-*rcsB*.



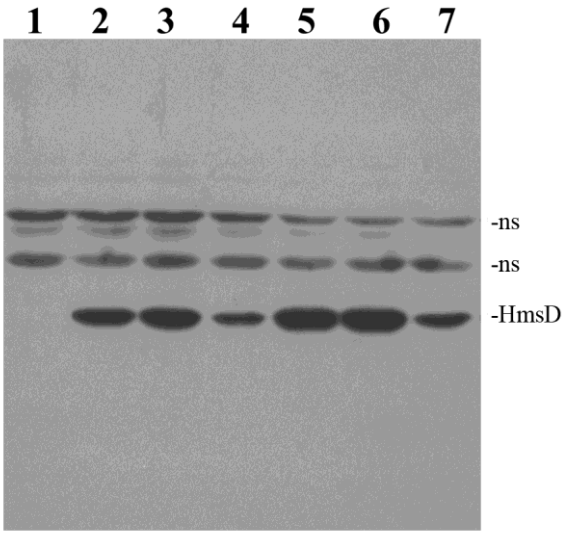
Supplementary Figure S5: Full-length blots for Figure 1c.



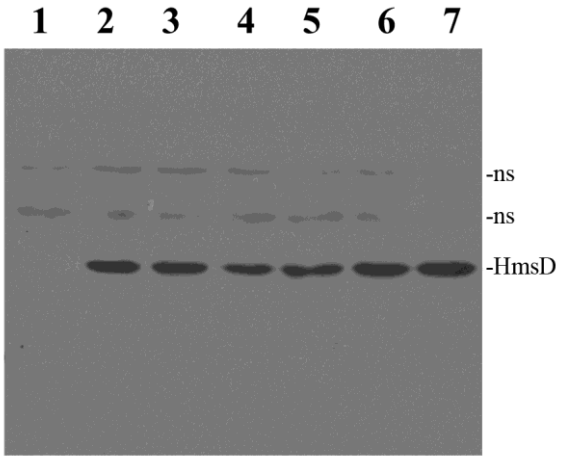
Supplementary Figure S6: Full-length blots for Figure 5a.



Supplementary Figure S7: Full-length blots for Figure 5b.



Supplementary Figure S8: Full-length blots for Figure 5c.



Supplementary Figure S9: Full-length blots for Figure 5d.

Supplementary References

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