The*Yersinia pestis* HmsCDE regulatory system is essential for blockage of the oriental rat flea (*Xenopsylla cheopis*), a classic plague vector

Alexander G. Bobrov, Olga Kirillina Viveka Vadyvaloo, Benjamin J. Koestler, Angela K. Hinz, Dietrich Mack, Christopher M. Waters, and Robert D. Perry

SUPPORTING INFORMATION

SUPPORTING TABLES

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Strains		
Escherichia coli		
DH5α	Cloning strain	Invitrogen
DH5α (λ *pir*)	Strain for maintenance of R6K origin suicide vectors	S. C. Straley
Yersinia pestis[a]		
KIM5 (pCD1Ap)+	Pgm^+ (Hms^+) Lcr^+(pCD1Ap; *yadA::amp*); pMT1, pPCP1; Ap^r	(Gong et al., 2001)
KIM5-2173.1 (pCD1Ap)+	Pgm^+ (Hms^+) *ΔhmsC2173.1* Lcr^{-+}(pCD1Ap; *yadA::amp*); pMT1, pPCP1; Ap^r	This study
KIM6+	Pgm^+ (Hms^+) Lcr^-; pMT1, pPCP1	(Fetherston et al., 1992)
KIM6-2051+	Pgm^+ Hms^- (*hmsT2051::*mini-*kan*) Lcr^-; pMT1, pPCP1; Km^r	(Jones et al., 1999)
KIM6-2090.2+	Pgm^+ Δ*hmsP2090.2* Lcr^-; pMT1, pPCP1	(Bobrov et al., 2011)
KIM6-2093+	Pgm^+ (Hms^+) *Δy2360::cam2093* Lcr^- ; pMT1, pPCP1; Cm^r	(Bobrov et al., 2011)
KIM6-2118	Pgm^+ Hms^- (Δ*hmsR2118*) Lcr^-; pMT1,	(Forman et al., 2006)

	pPCP1	
KIM6-2159.1+	Pgm$^+$ Δ*hmsD2159.1* Lcr$^-$; pMT1, pPCP1	(Bobrov et al., 2011)
KIM6-2173.1+	Pgm$^+$ Δ*hmsC2173.1* Lcr$^-$; pMT1, pPCP1	This study
KIM6-2173.2	Pgm$^+$ Hms$^-$ (Δ*hmsR2118*) Δ*hmsN2173.1* Lcr$^-$; pMT1, pPCP1	This study
KIM6-2173.3+	Pgm$^+$ Δ*hmsC2173.1* Δ*hmsT* Lcr$^-$; pMT1, pPCP1	This study
KIM6-2173.4 +	Pgm$^+$ Δ*hmsC2173.1* Δ*hmsT* Δ*hmsE::kan2174* Lcr$^-$; pMT1, pPCP1; Km^r	This study
KIM6-2174+	Pgm$^+$ Δ*hmsE::kan2174* Lcr$^-$; pMT1, pPCP1; Km^r	This study
KIM6-2174.1+	Pgm$^+$ Δ*hmsE 2174.1* Lcr$^-$; pMT1, pPCP1	This study
KIM6-2198+	Pgm$^+$ Δ*hmsCD2198* Lcr$^-$; pMT1, pPCP1	This study
KIM10+	Pgm$^+$ (Hms$^+$) Lcr$^-$; pMT1	(Perry et al., 1990)
CO92 [c]	Pgm$^+$ Lcr$^-$; pMT, pPCP	S.W. Bearden, CDC
CO92Δ*hmsC*	Pgm$^+$ Δ*hmsC2173.1* Lcr$^-$; pMT, pPCP	This study
Kimberley	Pgm$^+$ (Hms$^+$) Lcr$^-$; pMT, pPCP	(Fetherston et al., 1992)
Kuma	Pgm$^+$ (Hms$^+$) Lcr$^-$; pMT, pPCP	(Perry et al., 1990)
Kuma Δ*hmsC*	Pgm$^+$ Δ*hmsC2173.1* Lcr$^-$; pMT, pPCP	This study
Pestoides D	Pgm$^+$ (Hms$^+$) Lcr$^-$;pMT	S.W. Bearden, CDC
Pestoides D Δ*hmsC*	Pgm$^+$ Δ*hmsC2173.1* Lcr$^-$; pMT	This study
Pestoides F	Pgm$^+$ (Hms$^+$) Lcr$^-$; pMT	S.W. Bearden, CDC

Pestoides F Δ*hmsC*	Pgm^+ Δ*hmsC2173.1* Lcr^-; pMT	This study
Salazar	Pgm^+ (Hms^+) Lcr^-; pMT, pPCP	(Perry et al., 1990; Fetherston et al., 1992)
Yokohama	Pgm^+ (Hms^+) Lcr^-; pMT, pPCP	(Perry et al., 1990; Fetherston et al., 1992)
Plasmids		
pBAD30	Low copy number expression vector; *araC*$^+$; *araBAD* promoter; Ap^r	(Guzman et al., 1995)
pCP20	FLP recombinase expressing plasmid; Ap^r Cm^r	(Cherepanov and Wackernagel, 1995)
pKD3	Template plasmid; Cm^r	(Datsenko and Wanner, 2000)
pKD13	Template plasmid; Km^r	(Datsenko and Wanner, 2000)
pKD46	Red recombinase expressing plasmid; Ap^r	(Datsenko and Wanner, 2000)
pRMCD28-T5	*phoA* fusion vector with T5 promoter; Ap^r	(Bobrov et al., 2008)
pRMCD70-T5	*lacZ* fusion vector with T5 promoter; Ap^r	(Bobrov et al., 2008)
pSkippy	FLP recombinase expressing plasmid; Ap^r	(Price et al., 2012)
pWL204	Derivative of pKD46; Ap^r Sac^s	(Lathem et al., 2007)
pBAD-*hmsC*	658 bp fragment encoding *hmsC* cloned into pBAD30 by SmaI/XbaI; arabinose	This study

	inducible expression; Ap^r	
pBAD-*hmsE*	619 bp fragment encoding *hmsE* cloned into pBAD30 by EcoRI/SmaI; arabinose inducible expression; Ap^r	This study
pKNG-*ΔhmsR*	Fragment with *ΔhmsR* cloned into pKNG101; Sm^r Sac^s	(Forman et al., 2006)
pKNG-*ΔhmsT-Δscar*	Fragment with *ΔhmsT-Δscar* cloned into pKNG101; Sm^r Sac^s	(Bobrov et al., 2011)
pRMCD28-T5-*hmsC*-R207	*hmsC*-R207-*phoA* gene fusion cloned into pRMCD28-T5 by XbaI/PstI; Ap^r	This study
pRMCD70-T5-*hmsC*-R207	*hmsC*-207-*lacZ* gene fusion cloned into pRMCD70-T5 by XbaI/PstI; Ap^r	This study
pRMCD28-T5-*hmsD*-G425	*hmsD*-G425-*phoA* gene fusion cloned into pRMCD28-T5 by XbaI/PstI; Ap^r	This study
pRMCD70-T5-*hmsD*-G425	*hmsD*-G425-*lacZ* gene fusion cloned into pRMCD70-T5 by XbaI/PstI; Ap^r	This study
pRMCD28-T5-*hmsD*-R156	*hmsD*-R156-*phoA* gene fusion cloned into pRMCD28-T5 by XbaI/PstI; Ap^r	This study
pRMCD70-T5-*hmsD*-R156	*hmsD*-R156-*lacZ* gene fusion cloned into pRMCD70-T5 by XbaI/PstI; Ap^r	This study

[a] All *Y. pestis* strains lacking the pCD plasmid (Lcr$^-$) are avirulent. KIM5 strains were constructed by electroporation of pCD1Ap into KIM derivatives. KIM

derivatives with plus sign in possess an intact 102-kb chromosomal *pgm* locus which contains, among others, the *hmsHFRS* operon that is essential for biofilm formation. All KIM derivatives lacking a plus sign have either a *pgm* deletion or a mutation within the *pgm* locus.

[b] Abbreviations: Ap^r, Cm^r, Km^r, and Sm^r, respectively indicate resistance to ampicillin, chloramphenicol, kanamycin, and streptomycin.

[c] CO-99-3015 is the CDC stock of CO92. For simplicity, CO92 is used in the text.

Table S2. Primers used in this study.

Primer name	*Primer sequence (5'→3')*
Gene deletion	
Y3729 RED-1	CAACCACCACATTTAGCATCCCTTTTTAGCCCAACAGG AAGCGTAAAAGTGTAGGCTGGAGCTGCTTCG
Y3729 RED-2	TTCGGCTTCATCGTGCATTAGCCTTCTTACGGGCAAGT TGCAATACACATTCCGGGGATCCGTCGACC
Y3729 RED-2-pKD3	TCGGCTTCATCGTGCATTAGCCTTCTTACGGGCAAGTT GCAATACACTACATATGAATATCCTCCTTAGT
Y3731 RED-1	TGCCTATCAGCCGAATACGACTAACACAGAAAGTTTAG GATAGGTGACGTGTAGGCTGGAGCTGCTTCG
Y3731 RED-2	ACAGCCGCAAACCGGTAAATAAAGGACGTTAGGACGC GGTGATAATAAATTCCGGGGATCCGTCGACC
Y3730 RED-2	TTTGCATTTTCTGCATGTCACCTATCCTAAACTTTCTGT CATATGAATATCCTCCTTA
Control of deletion	
Y3729-1	TAGCCCAACAGGAAGCGTAA
Y3729-Xba-2	GATTCTAGATCGGCTTCATCGTGCATTAG
Y3730delProbeR	CACATCACTGTCGAATAGCACT
Cm-Alex1	AATATCCAGCTGAA*CG*GTCTG

HmsT-AO-1	GTGGTACAACATGCTGACGG
HmsT-AO-2	CACCAGAAAGGGGAAATGGG
HmsRO-XbaI	GCTCTAGACTCATGATTTCACCCTCCCAAT
HmsRO-EcoRI	GGAATTCTACTGGCGAACCACCGCTAAAAG
pQE3730R	CTAAGATCTTCCTAAACTTTCTGTGTTAGTCG
Km2	CAATAGCAGCCAGTCCCTTC
pQE3730F	CACCCATGGTATCACGGCGGATGTTGC
Km1	ACTGGGCTATCTGGACAAGG
Gene cloning	
Y3729-1	TAGCCCAACAGGAAGCGTAA
Y3729-XbaI-2	GATTCTAGATCGGCTTCATCGTGCATTAG
Y3731pBAD-EcoRI	GCCGAATTCGACTAACACAGAAAG
Y3731-down	GGCTCTGGGTCTATATCTGAG
Y3729-SD-fus-XbaI	ATCTCTAGATAGCCCAACAGGAAGCG
Y3729-R207-PstI	TTACTGCAGATCGTGCATTAGCCTTCTTACG
Y3730-SD-fus-XbaI	CAATCTAGACGTAAGAAGGCTAATGCACG
Y3730-G425-PstI	ATGCTGCAGATCCTAAACTTTCTGTGTTAGTCGTATTC
Y3730-R156-PstI	GTACTGCAGAACGCAATAAGCTGCCG
Sequencing	
hmsA_region_F	AGTGGCTCGCTTAGGTGG
hmsA_region_R	GCTTCGCCCTCAATCTCG
hmsA_region_Seq_F	CAACGAGCGGATGAAGCG
hmsA_region_Seq_R	GAGCCACGTTGAGTCTGC

References

Bobrov, A.G., Kirillina, O., Forman, S., Mack, D., and Perry, R.D. (2008) Insights into *Yersinia pestis* biofilm development: topology and co-interaction of Hms inner membrane proteins involved in exopolysaccharide production. *Environ Microbiol* **10**: 1419-1432.

Bobrov, A.G., Kirillina, O., Ryjenkov, D.A., Waters, C.M., Price, P.A., Fetherston, J.D. et al. (2011) Systematic analysis of cyclic di-GMP signalling enzymes and their role in biofilm formation and virulence in *Yersinia pestis*. *Mol Microbiol* **79**: 533-551.

Cherepanov, P.P., and Wackernagel, W. (1995) Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158**: 9-14.

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 6640-6645.

Fetherston, J.D., Schuetze, P., and Perry, R.D. (1992) Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element. *Mol Microbiol* **6**: 2693-2704.

Forman, S., Bobrov, A.G., Kirillina, O., Craig, S.K., Abney, J., Fetherston, J.D., and Perry, R.D. (2006) Identification of critical amino acid residues in the plague biofilm Hms proteins. *Microbiology* **152**: 3399-3410.

Gong, S., Bearden, S.W., Geoffroy, V.A., Fetherston, J.D., and Perry, R.D. (2001) Characterization of the *Yersinia pestis* Yfu ABC iron transport system. *Infection and Immunity* **67**: 2829-2837.

Guzman, L.M., Belin, D., Carson, M.J., and Beckwith, J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J Bacteriol* **177**: 4121-4130.

Jones, H.A., Lillard, J.W., Jr., and Perry, R.D. (1999) HmsT, a protein essential for expression of the haemin storage (Hms^+) phenotype of *Yersinia pestis*. *Microbiology* **145**: 2117-2128.

Lathem, W.W., Price, P.A., Miller, V.L., and Goldman, W.E. (2007) A plasminogen-activating protease specifically controls the development of primary pneumonic plague. *Science* **315**: 509-513.

Perry, R.D., Pendrak, M.L., and Schuetze, P. (1990) Identification and cloning of a hemin storage locus involved in the pigmentation phenotype of *Yersinia pestis*. *J Bacteriol* **172**: 5929-5937.

Price, P.A., Jin, J., and Goldman, W.E. (2012) Pulmonary infection by *Yersinia pestis* rapidly establishes a permissive environment for microbial proliferation. *Proc Natl Acad Sci U S A* **109**: 3083-3088.