

Supplementary Table S1. The strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	Reference
<i>E. coli</i>		
S17-1 λ <i>pir</i>	λ - <i>pir</i> lysogen of S17-1, <i>thi pro hsdR hsdM⁺ recA</i> RP4 2-Tc::Mu-Km::Tn7	(Simon, et al. 1983)
BL21(DE3)	Host for expression vector pET28a	Novagen
<i>Y. pseudotuberculosis</i>		
<i>Yptb</i> YPIII	Wild-type <i>Y. pseudotuberculosis</i> , Nal ^r	(Rosqvist, et al. 1988)
<i>Yptb</i> Δ <i>hpaR</i>	<i>hpaR</i> gene deleted in <i>Yptb</i> , Nal ^r	This study
<i>Yptb</i> Δ <i>hpaR</i> Δ <i>clpV4</i>	<i>hpaR</i> and <i>clpV4</i> gene deleted in <i>Yptb</i> , Nal ^r	This study
<i>Yptb</i> WT(Vector)	<i>Yptb</i> Wild-type containing pKT100, Nal ^r , Km ^r	(Zhang, et al. 2013)
<i>Yptb</i> Δ <i>hpaR</i> (Vector)	<i>Yptb</i> Δ <i>hpaR</i> containing pKT100, Nal ^r , Km ^r	This study
<i>Yptb</i> Δ <i>hpaR</i> (<i>hpaR</i>)	<i>Yptb</i> Δ <i>clpV4</i> containing pKT100- <i>hpaR</i> , Nal ^r , Km ^r	This study
<i>Yptb</i> Δ <i>hpaR</i> Δ <i>clpV4</i> (Vector)	<i>Yptb</i> Δ <i>hpaR</i> Δ <i>clpV4</i> containing pKT100, Nal ^r , Km ^r	This study
<i>Yptb</i> Δ <i>hpaR</i> Δ <i>clpV4</i> (<i>hpaR</i>)	<i>Yptb</i> Δ <i>hpaR</i> Δ <i>clpV4</i> containing pKT100- <i>hpaR</i> , Nal ^r , Km ^r	This study
Plasmid		
pKT100	Cloning vector, p15A replicon, Km ^r	(Hu, et al. 2009)
pKT100- <i>hpaR</i>	<i>hpaR</i> under the control of chloramphenicol resistance gene promoter in plasmid pKT100	This study
pET28a	Expression vector with N-terminal hexahistidine affinity tag, Km ^r	Novagen
pET28a- <i>hpaR</i>	pET28a carrying <i>hpaR</i> coding region, Km ^r	This study
pME6032	Shuttle vector, Tc ^r	(Zhao and

		Shao 2015)
pME6032- <i>yezP-vsvg</i>	pME6032 carrying <i>yezP-vsvg</i> coding region, Tc ^r	(Wang, et al. 2015)
pDM4	Suicide vector, <i>mobRK2</i> , <i>oriR6K</i> , <i>pir</i> , <i>sacB</i> , Cm ^r	(Milton, et al. 1996)
pDM4- Δ <i>hpaR</i>	Construct used for in-frame deletion of <i>hpaR</i> , Cm ^r	This study
pDM4- <i>hpaG1p::lacZ</i>	For <i>hpaG1</i> promoter fusion to <i>Yptb</i> , Cm ^r	This study
pDM4-T6SS4 <i>p::lacZ</i>	For T6SS4 promoter fusion to <i>Yptb</i> , Cm ^r	This study
pDM4-T6SS4 <i>pM::lacZ</i>	T6SS4 <i>pM::lacZ</i> , HpaR binding site mutated, Cm ^r	This study

*Nal^r, Cm^r, Km^r, Tc^r and Amp^r represent resistance to naladixic acid, chloramphenicol, kanamycin, tetracycline and ampicillin at 15, 30, 50, 10 and 100 µg/mL, respectively.

Supplementary Table S2. Primers used in this study.

Primers	5'-3' sequence	Function
<i>hpaR</i> -UF- <i>Xba</i> I	CTAGTCTAGAAATGGCCTACTTCCTTGCT GG	To generate pDM4- Δ <i>hpaR</i>
<i>hpaR</i> -UR	ACTCTTCATCCAGTGATTCATGCATAA GTA	
<i>hpaR</i> -DF	TGAATCACTGGATGAAGAGTGATGGG TTTA	
<i>hpaR</i> -DR- <i>Spe</i> I	CTAGACTAGTTTCTCAATCTTGATTGAG TT	
<i>hpaR</i> -F- <i>Bam</i> HI	CGCGGATCCATGCATGAATCACTGACCA T	To generate pKT100- <i>hpaR</i> , pET28a- <i>hpaR</i>
<i>hpaR</i> -R- <i>Sal</i> II	ACGCGTTCGACTCACTCTTCATCTTCTAA CA	
<i>HpaG1</i> -QF	ATGACTATGCCGTTTCGTGAC	qRT-PCR
<i>HpaG1</i> -QR	ATTGGAATATCCGCTTTAT	
<i>hpaE</i> -QF	TCCCTCACTGGGCTAACCTG	
<i>hpaE</i> -QR	TATCCTGCGTTTCCATCTGC	
<i>hpaB</i> -QF	GTGTCCAAGCGGATTTAGGT	
<i>hpaB</i> -QR	CTGCGAGATCGGGTAGATAA	
<i>vgrG4</i> -QF	AGGGAATCCATCCTACCA	
<i>vgrG4</i> -QR	AATTTGTCTTGCCGTTGC	
<i>hcp4</i> -QF	GTAAGTGTCTGGTGTCTCTCC	
<i>hcp4</i> -QR	CCATCAGGTTGCTGCTCT	
<i>clpV4</i> -QF	GGCGTCACCTTCTCCTATC	
<i>clpV4</i> -QR	TGAACCTCGCTGGTCTGT	
YPK_0997-QF	CACTGGAGGAAGGTGATTGG	
YPK_0997-QR	CGGGATTTATCATTGGCAGA	
YPK_2453-QF	GTGTCCAAGCGGATTTAGGT	
YPK_2453-QR	CTGCGAGATCGGGTAGATAA	
YPK_2458-QF	TTCAGCGAGTTCTGCCAAAT	
YPK_2458-QR	TTATCCCATCCCAGCATCCC	

YPK_3395-QF	CACGTCATGCCACGATTTGG
YPK_3395-QR	TTCTGCCTCGGTCATCAAGC
YPK_3402-QF	CGAACCGCAGGATAGTGAAA
YPK_3402-QR	TCCATGATCTCGACCGCACA
YPK_4175-QF	AGGCACGGTGGTATTAGAGG
YPK_4175-QR	GGAGCGATGTCCCATTTGTTT
YPK_2561-QF	CATACAGCCAATCCCACAGT
YPK_2561-QR	GGGCAGGCTAGGGTTAAAGG
YPK_3558-QF	GTATTGGGACGGTAGGAAGC
YPK_3558-QR	TTGAGCGCCGGTATAACTGA
YPK_3559-QF	CGGCATGAGTATGTGGAGCT
YPK_3559-QR	GCGTGGAGATAACCGTCTGG
YPK_3624-QF	TCTACACGCCAGACCCACAA
YPK_3624-QR	GCACCAAACCTCTGCCGCTAC
YPK_3776-QF	TTTAACTATGGGCTGGACTA
YPK_3776-QR	ATCAGGTAAGGCTGGAACAA
<i>16S RNA</i> -F	CTAGCGATTCCGACTTCAT
<i>16S RNA</i> -R	CCCTTATCCTTTGTTGCC

T6SS4 p -FP-F	AGAAGGCGTTGATGTTTGAC	HpaR Footprinting
T6SS4 p -FP-R	AACGCCGAATAATGCTTGAG	

Bio-T6SS4 p_{HpaR} -F	GTGGATTTCGCCTCAGGCAT	EMSA
Bio-T6SS4 p_{HpaR} -R	CATCCTGATTTACATACCTG	

T6SS4 p_{HpaR} -F	GTGGATTTCGCCTCAGGCAT
T6SS4 p_{HpaR} -R	CATCCTGATTTACATACCTG

T6SS4 p_{HpaR} -F- <i>Sa</i> I	ACGCGTCGACGTGGATTTCGCCTCAGGC AT	To generate pDM4- T6SS4 $p_{\text{HpaR}}::la$ <i>cZ</i>
T6SS4 $p_{\text{M}_{\text{HpaR}}}$ -F	ATTTGTTAGATTCCGAACCGTCATCGT GCTAATGGTTATG	

T6SS4 _{p_{HpaR}} M-R	GACGGTTCGGAATCTAACAAATAAGA GGACATAGATATGAGAAAGA	
T6SS4 _{p_{HpaR}} -R- <i>XbaI</i>	CTAGTCTAGACATCCTGATTTACATACC TG	
<i>hpaGlp</i> -FP-F	CTGCAATAATGCGATGGTCA	HpaR Footprinting
<i>hpaGlp</i> -FP-R	TTCAATGCAACGGCAAATAC	
<i>hpaGlp</i> -F	AAGTAACAACGACTCGACGT	EMSA
<i>hpaGlp</i> -R	GCAATGTTTCCTTTTGGCTTA	
<i>hpaGlp</i> -F- <i>SalI</i>	ACGCGTCGACCTGCAATAATGCGATGGT CA	To generate pDM4- <i>hpaGlp::lacZ</i>
<i>hpaGlp</i> -R- <i>XbaI</i>	CTAGTCTAGATTCAATGCAACGGCAAAT AC	

Underlined sites indicate restriction enzyme cutting sites added for cloning. Letters in boldface denote the annealing regions for overlap PCR.

Supplementary Table S3. Genes differentially transcribed in *ΔhpaR* mutant compared to the *Y. pseudotuberculosis* wild-type detected by RNA-seq.

CDS	Gene	Predicted function	^a Fold change
YPK_0033		hypothetical protein	2.64
YPK_0153		hypothetical protein	3.18
YPK_0442		cold shock protein	2.00
YPK_0774		hypothetical protein	2.11
YPK_0794		lipoprotein	1.65
YPK_0795		insertion element IS1 protein	3.11
YPK_0796		hypothetical protein	4.36
YPK_0833	<i>pilT</i>	twitching motility protein	1.65
YPK_0847		penicillin-binding protein 1C	7.63
YPK_0954		hypothetical protein	3.23
YPK_0997		chondroitin-sulfate-ABC endolyase/exolyase	1.66
YPK_0998		subtilase-type serine protease	2.09
YPK_1008		general secretion pathway protein H	3.86
YPK_1037		prepilin peptidase dependent protein A	2.74
YPK_1121		hypothetical protein	1.82
YPK_1160		hypothetical protein	1.87
YPK_1238		hypothetical protein	2.05
YPK_1244		hypothetical protein	7.18
YPK_1254		hypothetical protein	1.56
YPK_1317		protein of unknown function DUF943	1.61
YPK_1359		hypothetical protein	1.53
YPK_1395		hypothetical protein	4.17
YPK_1601		protein of unknown function DUF218	1.59
YPK_2452	<i>hpaC</i>	4-hydroxyphenylacetate-3-monooxygenase	2.31
YPK_2453	<i>hpaB</i>	4-hydroxyphenylacetate-3-monooxygenase	3.24
YPK_2454	<i>hpaX</i>	4-hydroxyphenylacetate permease	3.37
YPK_2455	<i>hpaI</i>	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase	3.83
YPK_2456	<i>hpaH</i>	2-oxo-hept-3-ene-1,7-dioate hydratase	3.91
YPK_2457	<i>hpaF</i>	5-carboxymethyl-2-hydroxymuconate isomerase	4.25
YPK_2458	<i>hpaD</i>	3,4-dihydroxyphenylacetate 2,3-dioxygenase	4.42
YPK_2459	<i>hpaE</i>	5-carboxymethyl-2-hydroxymuconic-	4.45
YPK_2460	<i>hpaG2</i>	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	4.56
YPK_2461	<i>hpaG1</i>	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	4.50
YPK_2494		hypothetical protein	1.53
YPK_2604		hypothetical protein	2.00
YPK_2893		hypothetical protein	1.58
YPK_2895		8-oxo-dGTP diphosphatase	7.34
YPK_3508		hypothetical protein	1.54
YPK_3098	<i>yscL</i>	type III secretion apparatus protein	2.71
YPK_3229	<i>comE</i>	competence protein	2.42
YPK_3294		transcriptional regulator, LysR family	2.04
YPK_3359	<i>dcuB</i>	anaerobic C4-dicarboxylate transporter	2.57
YPK_3395		L-ribulose-5-phosphate 4-epimerase	1.52

YPK_3397		DeoR family transcriptional regulator	2.56
YPK_3398		simple sugar transport system substrate-binding	1.80
YPK_3399		simple sugar transport system ATP-binding	2.59
YPK_3400		simple sugar transport system permease protein	2.02
YPK_3401		simple sugar transport system permease protein	2.28
YPK_3402		hexulose-6-phosphate isomerase	2.13
YPK_3403		L-xylulokinase	2.53
YPK_3414		hypothetical protein	1.95
YPK_3653		AI-2 transport system substrate-binding protein	1.88
YPK_3654		putative autoinducer-2 (AI-2) aldolase	1.87
YPK_3655		autoinducer 2-degrading protein	2.31
YPK_3748		hypothetical protein	6.19
YPK_3915		type III secretion system protein	2.91
YPK_4175		DNA ligase (NAD+)	1.60
YPK_0238	<i>tsgA</i>	MFS transporter	-3.88
YPK_0382		maltose operon periplasmic protein	-2.31
YPK_0678		tight adherence protein E	-3.54
YPK_0680		tight adherence protein C	-2.66
YPK_0681		tight adherence protein B	-4.20
YPK_0773		hypothetical protein	-1.56
YPK_0789		protocatechuate 3,4-dioxygenase	-1.61
YPK_0886		hypothetical protein	-6.07
YPK_1011		general secretion pathway protein K	-4.03
YPK_1013		hypothetical protein	-3.58
YPK_1269		3-phenylpropionic acid transporter	-2.03
YPK_1438		nucleoside transport protein	-3.33
YPK_1584		hypothetical protein	-5.59
YPK_1761	<i>hsf</i>	adhesin	-2.14
YPK_2286		putative transposase	-6.24
YPK_2297		GPW/gp25 family protein	-1.87
YPK_2316		hypothetical protein	-5.98
YPK_2341		phage baseplate assembly protein V	-2.83
YPK_2462	<i>hpaR</i>	MarR family transcriptional regulator	-11.55
YPK_2561		cytidine deaminase	-5.25
YPK_2588		hypothetical protein	-4.52
YPK_2720		iron complex transport system substrate-binding	-1.93
YPK_3534			-4.74
YPK_3550	<i>impL</i>	type VI secretion system protein	-1.52
YPK_3551	<i>impK</i>	type VI secretion system protein	-1.53
YPK_3552	<i>impJ</i>	type VI secretion system protein	-1.63
YPK_3555		hypothetical protein	-1.77
YPK_3556		adenylate cyclase	-1.74
YPK_3557		adenylate cyclase	-1.77
YPK_3558	<i>vgrG</i>	type VI secretion system secreted protein	-1.78
YPK_3559	<i>vasG</i>	type VI secretion system protein	-1.70
YPK_3560	<i>impH</i>	type VI secretion system protein	-1.52
YPK_3561	<i>impG</i>	type VI secretion system protein	-1.79
YPK_3564	<i>impC</i>	type VI secretion system protein	-1.77
YPK_3565	<i>impB</i>	type VI secretion system protein	-1.69

YPK_3566	<i>impA</i>	type VI secretion system protein	-1.90
YPK_3623		membrane protein	-1.76
YPK_3624		purine-nucleoside phosphorylase	-3.62
YPK_3625		phosphopentomutase	-4.05
YPK_3626		thymidine phosphorylase	-4.38
YPK_3627		deoxyribose-phosphate aldolase	-4.05
YPK_3628		concentrative nucleoside transporter, CNT	-2.70
YPK_3670		hypothetical protein	-2.14
YPK_3724		ATP-dependent RNA helicase	-1.60
YPK_3776		2',3'-cyclic-nucleotide 2'-phosphodiesterase	-3.63
YPK_3844		phosphoribosylaminoimidazol (AIR) synthetase	-7.68
YPK_3912		type III secretion system protein	-5.56
YPK_3949		protein-tyrosine phosphatase	-2.77
YPK_3950		uridine phosphorylase	-4.87
YPK_4107		hypothetical protein	-2.32

RNA-seq-based transcriptomics analysis was performed using total RNAs isolated from *Y. pseudotuberculosis* YPIII $\Delta hpaR$ mutant compared to the *Y. pseudotuberculosis* YPIII wild-type. The genes that are at least 1.5-fold changed in biological replicates were considered as significant. T6SS4 genes and the *hpa-meta* gene were highlighted in yellow. qRT-PCR verified genes were shown in boldface. ^a Fold change was defined by $2^{(\text{the gene expression ratio of } \Delta hpaR \text{ mutant to the } Y. pseudotuberculosis \text{ YPIII wild-type})}$.

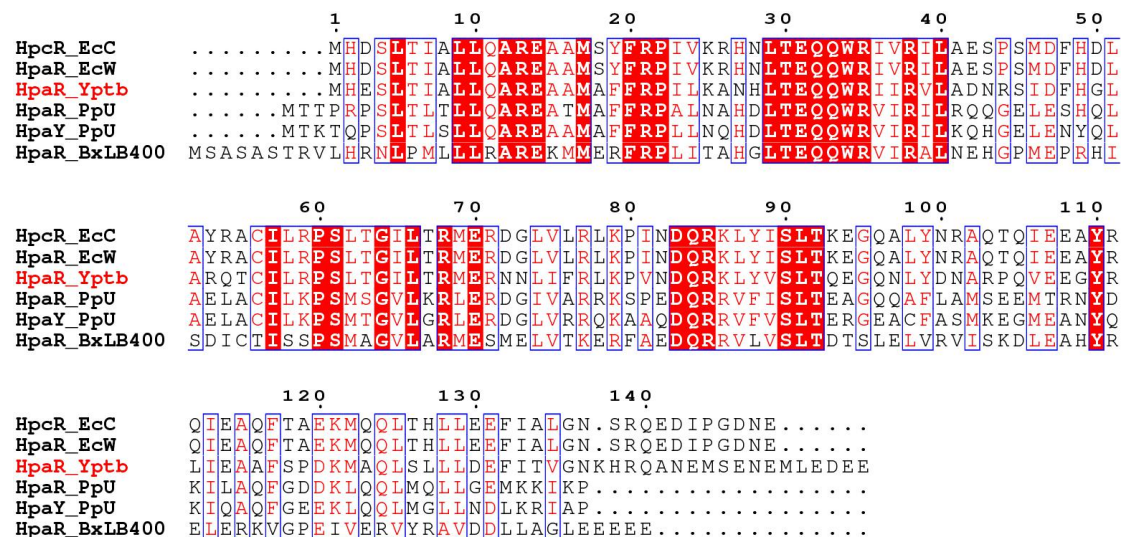


Fig. S1. Protein sequence alignment of HpaR homologues by CLUSTAL W.

The sequences used in alignment have been deposited in the GenBank database (Accession No. EcC *Escherichia coli* C (S56952.1), EcW *Escherichia coli* W (Z37980.2), Yptb *Yersinia pseudotuberculosis* YPIII (ACA68739.1), PpU *Pseudomonas putida* U (FJ904934.1), BxLB400 *Burkholderia xenovorans* LB400 (ABE33958.1)). The result was exported by ESPript (<http://esprict.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>).

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