Supplementary material for:

Common and divergent features in transcriptional control of the homologous small RNAs GImY and GImZ in *Enterobacteriaceae*

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Proteus mirabilis Edwardsiella ictaluri Erwinia tasmaniensis ET Erwinia pyrifoliae EP1/96	1/99 - hemX	glmZ t	
Klebsiella pneumoniae, E Salmonella sp., Citrobac Yersinia pseudotuberculo Providencia alcalifaciens,	Enterobacter cancerogenus, ster koseri, ssis YPIII, Y. enterocolítica hemX , P. rustigianii	hemY	
Photorhabdus asymbiotic	a hemX		
Photorhabdus luminescei	ns hemX		
Yersinia pestis Antiqua	hemX		
Enterobacter sp. 638 Serratia proteamaculans	hemX		
Shigella boydii Sb227 Shigella sonnei		hemY	
Escherichia coli (O26:H11	1, SE11)	hemY	
Shigella flexneri	hemX		
Escherichia coli (APEC 0 Escherichia albertii Shigella dysenteriae Sd1	11, K-12, O157:H7, UTI89) 97	hemY	
Escherichia fergusonii	hemX hemY		
Cronobacter turicensis Cronobacter sakazakii AT	TCC BAA-894		
Erwinia carotovora SCRI	1043 - hemX		
<i>Dickeya zeae</i> Ech1591	hemX		
<i>Dickeya dadantii</i> Ech586	hemX V		
Pectobacterium wasabiae Pectobacterium carotovo	e rum <u>hemX</u>	hemY	(_gntR"prpD"cdshisJbatBhisM
	abgB : putative peptidase araC : AraC-type transcriptional regulator as/A : arylsulfatase as/B : sulfatase maturation enzyme batB : amino acid ABC transporter, membrai dc3 : hypothetical protein COG4673 : protein involved in phenol degra COG4667 : predicted setterase of the alnha-1	ne subunit dation peta hydrojąse superfam	osmY : predicted periplasmic or secreted lipoprotein prfC : peptide chain release factor RF-3 prpD : uncharacterized protein involved in propionate catabolism purB : probable lyase rffM = wecG : UDP-N-acetyI-D-mannosaminuronic acid transferase rffT = wecF : Fuc4Nac (4-acetamido-4,6-dideoxy-D-galactose) transferase rim1 : putative acetyItransferase liv rsrmc : 16S RNA G1207 methylase
2	ECA4198 : putative exported phosphatase glaA : putative apicorol dehydrogenase glnQ : putative amino-acid ABC transporter / gnR : GnR-type transcriptional regulator hisJ : putative amino-acid ABC transporter holD : DNA polymerase III, psi subunit lysR : LysR-type transcriptional regulator	ATP-binding protein inding protein permease protein	Ital:: predicted voidoreductase (related to aryl-alcohol dehydrogenases) IaD:: Mg-dependent DNase Inp:: transposase wzyE: O-antigen translocase (flippase) wzyE: = nfT: wzyE: = nfT: wzyF: = nfT: wzyE: > nino acid transporter : pseudogene/ truncated gene

Fig. S1. Gene synteny of the *glmZ* **region in** *Enterobacteriaceae.* Information about gene co-localization and annotation was retrieved using the MicrobesOnline (1) and KEGG (2) databases. Genes are just approximately drawn to scale.

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Escherichia coli Shigella sp. Citrobacter koserii	gln	nY
Escherichia albertii		girk - yfnG girR yginB hmp glyA - yphH -
Enterobacter sp. 638 Enterobacter cancerogenus Cronobacter sakazakii ATCC BA Klebsiella pneumoniae	A-894	
Cronobacter turicensis		girk ying girk ying fire ying yay
Erwinia tasmaniensis ET1/99		
Erwinia pyrifoliae ET1/96		girk /yhg girR /ginE giyA / csiE g663
Erwinia carotovora SCRI1043 Pectobacterium wasabiae Pectobacterium carotovorum	mttF purL	girk yrthG girR ginB (pemB paey hmp -
<i>Dickeya dadantii</i> Ech586 <i>Dickeya zeae</i> Ech1591		
Escherichia fergusonii		girk Jufr.G girk JginB adk Jhemit aes
Salmonella sp.		
Proteus mirabilis		girk yring girk nade ginb cds thmp give h
Yersinia pseudotuberculosis 🛛 🛪		
Yersinia pestis Antiqua		f1] girk Jufting girk nade gine Kods the hope give for the give for t
Yersinia enterocolitica		girk yrthG girk node ginb yrth cas (php (yrth)
Serratia proteamaculans		girk yrfnG girR hende ginB yrysR putA
Photorhabdus asymbiotica		
Photorhabdus luminescens		
Edwardsiella ictaluri		girk / yfhG girR / nade / ginB / gmhA / COG121
Providencia alkalifaciens Providencia rustigianii		
	adk : adenylate kinase aes : acetylesterase cadA : lysoine decarboxylase cadA : lysoine decarboxylase cadB : hysoinetical protein c/B : response regulator with CheY-like receiver domain COC121 : predicted glutamine amidotransferase COG1789 : SoxR-type transport system, periplasmic component cCG3683 : ABC-type transport system, periplasmic component cslE : stationary phase-inducible protein <i>ehpF</i> : putative AMP-dependent synthetase/ligase glnB : protein PII glyA : serine hydroxymethyltransferase <i>hemH</i> : ferrochelatase <i>hmp</i> : flavohemoglobin	lysR : LysR-type transcriptional regulator mtlF = yfhD : membrane-bound lytic murein transglycosylase F nadE : glutamine-dependent NAD(+) synthetase paeY : peciti accyliseterase pelB : pecitale lyase pelB : pecitale lyase pmB : peciticet metal-dependent hydrolase puL : phosphoribosylformylglycinamide synthetase puL1 : aldehyde dehydrogenase trp : transposase uhfT : putative integral membrane protein yhfT : predicted main acid racemase yidX : peptide:H* symporter yhA : DocX family protein ypHA : NagC-liko transcriptional regulator

Fig. S2. Gene synteny of the *glmY* **region in** *Enterobacteriaceae.* Information about gene co-localization and annotation was retrieved using the MicrobesOnline (1) and KEGG (2) databases. Genes are just approximately drawn to scale.

Pantnea ananalis IMG 20103 Erwinia amyonora CEPI-1430 Pacubacterium carcotorium subsp. artrospitica SCR1043 Pacubacterium carcotorium subsp. artrospitica SCR1043 Pacubacterium vasabiae WPP183 Pacubacterium vasabiae WPP183 Sarratia proteamacularis SB8 Sarratia proteamacularis SB8 Concolacter software SC02 Concolacter software SC02 Satrocella anterica servor Typhys str. CT18 Satrocella anterica servor Typhys str. CT18 Satrocella anterica SU52 Satrocella anterica SC03 Satrocella anterica SC03 S	Pennee ananatis LNC 20103 Erwinia tamuniostis E1193 Percibicarieum carcitovum subsp. arrosoptice SCR11043 Percibicarieum carcitovum subsp. arrosoptice SCR11043 Percibicarieum vaschieum PC1 Assenghonus nasoniae Protorhabdus lumandus saynibidica Sarratia odorfina 4Rx13 Sarratia odorfina 4Rx13 Sarratia odorfina 4Rx13 Sarratia narexocaliza SB8 Concolearte turinesis 2032 Concolearte subsp. famocolitica 8081 Yersinia percolarita SCR2 Concolearte subsp. famocolitica 8081 Concolearte subsp. famocolitica 8081 Concolearte functiona 91001 Versinia percolarita FICC BAA-894 Concolearte service Tarbity ACC 5150 Satimonella anterica servica Tarbity Att. ATCC 5150 Satimonella anterica servica Tarbity Att. ATCC 5157 Satimonella anterica servica Tarbity Att. TCR 5569 Escherichia ford 71717 8st. 1224865 Siglial ageanteriae SUS9 Escherichia ford 73 st. 30 Siglial ageant 23 st. 30 Siglial ageant 24 st. 30 Siglial ageant 25 st. 30 Siglia ageant 25 st. 30 Siglia ageant 35 st. 30 Siglia a
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B. 96. 901- Construction of the second sec	AG 2000 THE ANALY
	HHF-site 1 Control transmission
G ^{TD} promoter -35 -6" promoter -35 -2 -24 TTGAC -24 -24	-1.10 -1.10

Fig. S3. Sequence alignment of the *glmY* promoter regions from 39 enterobacterial genomes. Fully conserved nucleotide positions are highlighted in red, while residues conserved in the majority of these sequences are in blue. The transcriptional start site of *glmY* is marked with an arrow. The GIrR binding sites (ABS), putative IHF binding sites and the -24/-12 sequence motifs of σ^{54} promoters are boxed. The -35/-10 sequence motifs of overlapping σ^{70} -promoters are also boxed. The respective consensus sequences are shown above the alignment. Sequences were compiled from the following genomes (accession numbers are in parentheses): *Pantoea ananatis* LMG 20103 (NC 013956.1), *Erwinia tasmaniensis* Et1/99

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(NC 010694.1), Erwinia amylovora CFBP 1430 (NC 013961.1), Pectobacterium carotovorum subsp. atroseptica SCRI1043 (NC 004547.2), Pectobacterium carotovorum subsp. carotovorum PC1 (NC 012917.1), Pectobacterium wasabiae WPP163 (NC 013421.1), Arsenophonus nasoniae (FN545167.1), Photorhabdus luminescens subsp. laumondii TTO1 (NC 005126.1), Photorhabdus asymbiotica (NC 012962.1), Serratia Serratia (NC 009832.1), odorifera proteamaculans 568 4Rx13 (NZ ADBX01000009.1), Serratia marescens Db11 [http://www.sanger.ac.uk], Proteus mirabilis HI4320 (NC 010554.1), Yersinia pseudotuberculosis YPIII (NC 010465.1), Yersinia pestis biovar Microtus str. 91001 (NC 005810.1), Yersinia enterocolitica subsp. enterocolitica 8081 (NC 008800.1), Yersinia bercovieri ATCC 43970 (NZ_AALC02000019.1), Dickeya zeae Ech1591 (NC 012912.1), Dickeya dadantii Ech586 (NC 013592.1), Cronobacter turicensis z3032 (NC 013282.1), Cronobacter sakazakii ATCC BAA-894 (NC 009778.1), Citrobacter koseri ATCC BAA-895 (NC 009792.1), Citrobacter rodentium ICC168 (NC 013716.1), Enterobacter sp. 638 (NC 009436.1), Salmonella enterica subsp. enteric serovar Choleraesuis str. SC-B67 (NC 006905.1), Salmonella enterica subsp. enteric serovar Paratyphi A str. ATCC 9150 (NC 006511.1), Salmonella enterica subsp. enterica serovar Typhi str. CT18 (NC 003198.1), Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 (NC 003197.1), Klebsiella pneumoniae 342 (NC 011283.1), Klebsiella pneumonia subsp. pneumoniae MGH 78578 (NC 009648.1), Escherichia fergusonii ATCC 35469 (NC 011740.1), Escherichia albertii TW07627 (NZ ABKX01000003.1), Shigella sonnei Ss046 (NC 007384.1), Shigella dysenteriae Sd197 (NC 007606.1), Escherichia coli O127:H6 str. E2348/69 (NC 011601.1), Shigella boydii Sb227 (NC 007613.1), Shigella flexneri 2a str. 301 (NC 004337.1), Escherichia coli O157:H7 str. EC4486 (NZ ABHS01000009.1), Escherichia coli K12 str. MG1655 (U00096.2). The alignment was compiled using the AlignX tool of software Vector NTI Advance[™] 9.0.

Kiebseille preurinnie Subp. preumoniae MGH 78578 Escherichia flegrusoni //TCC 35469 Escherichia flegrusoni //TCC 35469 Shigelia asonne Sa546 Escherichia coli 0127:145 str. E2349/69 Shigelia fleoreni 2a ett. 301 Escherichia coli 0137:147 str. EC4486 Escherichia coli 0157:147 str. EC4486 Escherichia coli 0127:147 str. EC4486 Escherichia coli 0127:147 str. EC4486 Consensus of alignment:	Pecidibacterium carchorum subsp. arrospitica SCR1003 Pecidibacterium reactionum subsp. arrospitica SCR103 Pecidibacterium avasabie WPP163 Arsemptionus nasine Protorinadus luminescens subsp. lumnontii TTO1 Protoria periodibacterium et al. Serralia proteamaculans 58 Serralia proteamaculans 58 Serralia proteamaculans 58 Serralia proteamaculans 58 Corrobacter traffic subsp. futurnati 91001 Vinerinia periodibacturia et 91001 Vinerinia periodibacturia et 91001 Vinerinia periodibacturia et 91001 Vinerinia periodibacturia et 91001 Dickeya zae ELS191 Corrobacter traffic subsp. futurnati 91001 Dickeya zae ELS191 Corrobacter traffic Static Static Corrobacter traffic Static Static Corrobacter traffic servora Choleneaula str. SC-B67 Satironella enterica servora Typhimulum str. IL7 Satironella enterica servora Typhimulum str. IL7	Klabsielle pneumonies 342 Klabsielle pneumonies 342 Escherichie forgusoni ATCC 55489 Escherichie albeit TW07527 Shigelia sonrei 55416 Shigelia fastrari es 5419 Escherichie odi G157147 st. EC34866 Shigelia fastrari 2a st. 301 Escherichie odi G157147 st. EC4486 Escherichie odi G157147 st. EC4486 Escherichie odi G157147 st. EC4486 Escherichie odi G157147 st. EC4486 Escherichie odi G219143 Escherichie odi G219143	Serralia manascensa DB11 Protess matalia: H4200 Yessiha pesutodubercubesis PTIII Yessiha pesutoduber Kircutes \$1001 Yessiha persetu harvan ATCC 43970 Dickoya adamta PEATS02 Corrobacter Indensitis 2002 Corrobacter Indensitis 2002 Salmonella enforts ascross Typbinutium st. LTZ Consensus of alignment:	Pantoea ananalis LMG 20103 Envina amytorea CFBP1430 Envina amytorea CFBP1430 Pedcobactinum aerotovorum subsp. antroseptica SCR11043 Pedcobactinum exercitivorum subsp. antroseptica SCR11043 Pedcobactinum exercitivorum Subsp. autorovorum PC1 Pedcobactinum exercitivorum Subsp. autorovorum PC1 Pedcobactinum exercitivo PP163 Pedcobactinum exercitivo Statu Asemptinus aproleanaeuluita S68 Serratia profeanaeuluita S68 Serratia profeanaeuluita S68
0.1 0.1 0.2 0.2 0.3 0.4 0.1 <td>$\begin{array}{c} \operatorname{chr} \operatorname$</td> <td>$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \end{array} \end{array} \end{array} \\ \end{array} \\$</td> <td></td> <td></td>	$ \begin{array}{c} \operatorname{chr} \operatorname$	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \end{array} \end{array} \end{array} \\ \end{array} \\ $		

Fig. S4. Sequence alignment of the *glmZ* promoter regions from 39 enterobacterial genomes. The sequences classified into two groups, which exhibited no significant homologies to each other and are therefore shown in separate alignments. Fully conserved nucleotide positions within each group are highlighted in red, while residues conserved in the majority of sequences are in blue. Refer to legend to Fig. S3 for additional information. The same genome sequences as in Fig. S3 were used.



Fig. S5. *Y. pseudotuberculosis* **GIrR binds the** *glmY* **promoters of** *Y. pseudotuberculosis* **and** *E. coli.* EMSAs to test binding of *Y. pseudotuberculosis* GIrR protein to the *glmY* promoter regions of *Y. pseudotuberculosis* (-257 to +22) and *E. coli* (-238 to +22). The DNA fragments were obtained by PCR using the same primers as for construction of the corresponding *glmY'-lacZ* fusions tested in Fig. 2 B. In addition to the *glmY* promoter fragments, 200 bp (panel 1) or 400 bp DNA fragments (panel 2) covering the *lacZ* promoter were present as internal controls. The sizes of of the DNA size standard are given on the left.



Fig. S6. *Y. pseudotuberculosis* **GIrR** binds the cognate *gImZ* promoter, while the *E. coli gImZ* promoter is not bound. EMSAs to test binding of *Y. pseudotuberculosis* GIrR protein to the *gImZ* promoter regions of *Y. pseudotuberculosis* (-303 to +22) and *E. coli* (-424 to +32). The DNA fragments were obtained by PCR using the same primers as for construction of the corresponding *gImZ'-lacZ* fusions used in Fig. 3 B.

Α

Fig. S7





Fig. S7. Expression of glmY and glmZ in Y. pseudotuberculosis. (A) glmY and glmZ are expressed in Y. pseudotuberculosis. β -Galactosidase activities of Y. pseudotuberculosis carrying a glmY'-lacZ (column 2) or glmZ'-lacZ fusion (column 3) on a plasmid (pYG1 and pYG2, respectively). Cells carrying the empty *lacZ* fusion vector pKEM04 served as background control (column 1). (B) Stimulation of glmY and glmZ expression by GlrR in Υ. pseudotuberculosis. Additional plasmids carrying either glrR from Υ. pseudotuberculosis (plasmid pYG6, columns 2, 5) or E. coli (plasmid pBGG223, columns 3, 6) or no gene (empty vector pBAD18-cm, columns 1, 4) under PAra promoter control were introduced into Y. pseudotuberculosis carrying either the glmY'-lacZ fusion plasmid pYG1 (columns 1-3) or the glmZ'-lacZ fusion plasmid pYG2, respectively (columns 4-6). For the induction of glrR expression 0.2% arabinose was added and subsequently the βgalactosidase activities were determined.



Fig. S8. Binding of the *Y. pseudotuberculosis* **GIrR protein to its cognate** *glmZ* **promoter requires three activator binding sites.** EMSAs to monitor binding of *Y. pseudotuberculosis* GIrR to DNA fragments covering the *Y. pseudotuberculosis glmZ* promoter (-303 to +22). DNA fragments were tested that carried mutations within ABS3 (top, right) or in all ABS simultaneously (bottom, left). These DNA fragments were obtained by PCR using the corresponding *glmZ'-lacZ* fusions, presented in Fig. 4 A, as template and primers BG700/BG701. In addition, a truncated DNA fragment lacking all ABS was tested (-170 to +22; bottom, right). The DNA fragment was obtained using the primer pair BG755/BG701.



Fig. S9. Binding of IHF to the *E. coli gImY* and the *Y. pseudotuberculosis gImZ* promoter regions as revealed by EMSA. The DNA fragments were obtained by PCR making use of the primer pairs BG377/BG456 and BG700/BG701, respectively. As a difference to the experiments shown in Fig. 6 B, a DNA fragment covering the *ptsG* promoter (P_{ptsG}) from *Bacillus subtilis* was used as internal control rather than a *lacZ* promoter fragment. The P_{ptsG} fragment was amplified from the *B. subtilis* chromosome using primers IL5 (3) and JS11 (4).

	gImZ	glmY	glmZ	glmY
Consensus of alignment:	Erwinia amylovora CFBP1430 Peotobaderium aordovorum PC1 Photomabdus luminessens subsp. laumondii TT01 Yersinia pseudouberuulosi VPIII Serratia marescens Db11 Serratia marescens Db11 Cronobader sakazaii/ ATCC BAA894 Enterobaders 9,638 Citrobacter koser/ATCC BAA895 Safronalia enterica server Typhimurium str. LT2 Escherichia abertii TW07627 Escherichia eduetti TW07627 Escherichia eduetti 20197 Escherichia edugusonii ATCC 35469 Sintonalise Anternae St197 Singela dysenteriae St197	Citcbecter koseri ATCC BAA-895 Salmonella enterica serover Typhimurium str. LT2 Escherichia abertii TW07g27 phimurium str. LT2 Escherichia debertii TW07g27 Shigella dysenteriae S119 Escherichaeter sp. 638 Cronobaeter saczakii ATCC BAA-894 Erwinia amytovora CFBP1430 Protorhaetus uninessens subsp. laumondii TTO1 Pectobacterium carciovorum subsp. carotovorum PC1 Serrata marescens Db11 Serrata marescens Db11	Ewinia amplovora CFBP1430 Perciobacterium carotovoura subsp. carotovouram PC1 Pichothabdus luminessens subsp. laumondii TTO1 Kersinia pseudotubersulsis VPIII Corrobacter sakazakii ATCC BAA-894 Enterobacter sakazakii ATCC BAA-895 Citrobacter Koseri ATCC BAA-895 Salmonella enterica servoar Tryphimurium str. LT2 Escherichia abbertii TW07827 Escherichia disertii TW07827 Escherichia disertii TW07827 Escherichia fergusorii ATCC 35469 Kiebsiella preumoniee subsp. pneumoniae MGH7857 Consensus of alignment:	Citrobacter koseri ATCC BAA-885 Seirnonelle enterica serovar Typhimunium str. LT2 Escherichia abertii TW07627 Shigella dysenteriae S1937 Shigella dysenteriae S1937 Klebsielle pneumoniae subsp. pneumoniae MGH7857 Enterobacter sp. 538 Cronobacter saacakii ATCC BAA-834 Erwinia amytovora CFBP1430 Photorhabdus turinessens subsp. laurondii TTO1 Pectohabdus turinessens subsp. carotovorum PC1 Serratia marescens Db11 Serratia marescens Db11
G C TATCA AC COCCCAAACOTTCACTCACCACCCC TT TT C AACACA CTC AC COTOCT G A G TITTITT <i>g/mS</i> binding site		130 140 150 160 170 180 190 200 200 200 253 GER-G	$ \begin{array}{llllllllllllllllllllllllllllllllllll$	DIBURD — ПОДО ПОДО ПОДО ПОДО ПОДО ПОДО ПОДО ПО

Fig. S10. Sequence alignment of *glmY* and *glmZ* genes of 14 enterobacterial species. Fully conserved nucleotide positions are highlighted in red, while residues conserved in the majority of sequences are in blue. The positions involved in GlmZ/*glmS* interaction (according to (5,6)) are boxed. Refer to legend to Fig. S3 for accession numbers of the genome sequences used. The alignment was compiled using the AlignX tool of software Vector NTI AdvanceTM 9.0.

Fig. S11



Fig. S11. Phylogenetic tree of *gImY* and *gImZ* genes of 14 enterobacterial species. The tree was calculated from the sequence alignment shown in Fig. S10 using the AlignX tool of software Vector NTI AdvanceTM 9.0. The tree is built using the Neighbor joining method (7), which works on a matrix of distances between all possible sequence pairs. The calculated distance values, which are related to the degree of divergence between the sequences, are shown in parentheses.





Fig. S12. Phylogenetic tree of *glmY***- and** *glmZ***-promoter regions of 14 enterobacterial species.** The tree was calculated from a sequence alignment (data not shown) comprising the promoter sequences of the *glmY*- and *glmZ*-genes used for Fig. S11. The used sequences are shown in Figures S3 and S4, but the sequences downstream of the transcriptional start sites of the sRNAs were omitted. See Fig. S11 for additional information.

SUPPLEMENTAL "MATERIALS AND METHODS"

Construction of plasmids

For construction of the fusions of Y. pseudotuberculosis glmY (-257 to +22) and qlmZ (-303 to +22) to lacZ, the corresponding qlmY-5' and qlmZ-5' regions were amplified from the Y. pseudotuberculosis chromosome using the primer combinations BG698/BG699 and BG700/BG701, respectively. The PCR fragments were subsequently used to replace the Sall-Xbal fragment in plasmid pKES15, which yielded plasmids pYG1 and pYG2, respectively. To obtain a Y. pseudotuberculosis glmZ'-lacZ fusion carrying a mutated ABS1, this mutation was introduced by PCR using forward primer BG747 together with BG701. Insertion of this fragment between the Sall/Xbal-sites of pKES15 resulted in plasmid pYG9. Mutations in ABS2 and ABS3 were introduced by multiple mutation reaction (MMR; (8)). To this end, the 5'-phosphorylated oligonucleotides BG748 and/or BG754 carrying the mutations in ABS2 and ABS3, respectively, were used in addition to the forward primers BG700 or BG747 (mutation of ABS1) and reverse primer BG701 in PCRs. These PCRs contained thermo-stable Ampligase (Epicentre), which incorporated the mutagenesis primers during amplification. Insertion of the PCR fragments between the Sall/Xbal-sites of pKES15 yielded plasmids pYG10 (ABS2 mutated), pYG11 (ABS3 mutated) and pYG12 (all ABS mutated). The glmY-5' (-242 to +22) and glmZ-5' (-242 to +22) regions of S. typhimurium were amplified from chromosomal DNA using the primer combinations BG750/BG751 and BG752/BG753, respectively, and the PCR fragments were inserted between the Sall/Xbal sites of plasmid pKES15 to yield plasmids pYG7 and pYG8. Plasmids pBGG390 and pBGG391 are isogenic with plasmids pBGG201 and pBGG209, but carry mutations within the putative IHF1-site in the E. coli glmY upstream region. Plasmid pBGG390 was constructed by MMR using pBGG201 as template, BG377 and BG456 as external primers and the phosphorylated mutagenesis primer BG684. The resulting PCR fragment was cloned via Sall/Xbal into plasmid pKES15. To introduce the mutation in the -10 sequence, the AfIII-SacI fragment of pBGG390 was replaced by the corresponding fragment of pBGG209 resulting in plasmid pBGG391. The plasmids carrying the gradually 5'-truncated E. coli glmZ'-lacZ fusions were also constructed by cloning PCR fragments that were amplified from the E. coli chromosome between the Sall/Xbal sites of pKES15. The PCR fragments were obtained using reverse primer BG202 and one of the following forward primers resulting in the plasmid as indicated in parentheses: BG200 (pBGG111), BG333 (pBGG112), BG334 (pBGG113), BG335 (pBGG114), BG411 (pBGG170). Plasmid pBGG135 carrying the almZ'(-11 to +32)-lacZ fusion was constructed by ligation of hybridized 5'phosphorylated oligonucleotides BG347 and BG348 with the Sall/Xbaldigested vector pKES15. Hybridization was achieved by heating 150 µl of a solution containing 20 pMol of each oligonucleotide, 10 mM Tris/HCl pH 7.5 and 1 M NaCl to 99°C followed by slow cooling and precipitation with ethanol.

Plasmids pBGG157 and pBGG171 carry mutated -35 and -10 sequences in the context of the glmZ'(-40 to +32)-lacZ fusion, respectively. The mutations were introduced by forward primers BG388 and BG412, respectively, in PCRs using BG202 as reverse primer. The PCR fragments were subsequently inserted between the Sall/Xbal sites of plasmid pKES15. For construction of plasmid pBGG397 carrying Y. pseudotuberculosis glrR::His10 under tacOP control, glrR was amplified from the Y. pseudotuberculosis chromosome using primers BG696 and BG697. Subsequently, the PCR product was inserted between the Ndel- and Xbal-sites on plasmid pKES170. For construction of plasmid pYG6 carrying Y. pseudotuberculosis glrR under P_{Ara} promoter control, *qlrR* was amplified using primers BG727/BG728 and cloned between the Sacl and Xbal sites on plasmid pBAD18-cm. To construct plasmid pBGG389, which carries E. coli glrR under PAra control, the Sacl-HindIII fragment of plasmid pBGG223 encompassing the glrR gene was cloned between these sites on plasmid pBAD33. To obtain the isogenic plasmids pBGG398 and pBGG399, which code for the glrR-D56A and glrR-D56E alleles, MMRs were carried out using pBGG223 as template, the external primers BG490/BG491 and the mutagenesis primers BG685 and BG686, respectively. The MMR fragments were subsequently cloned between the Sacl/Xbal sites on plasmid pBAD33.

SUPPLEMENTAL TABLES

TADLE 0		
Name	Genotype or relevant structures ^a	Reference or construction
pBAD18-cm	P _{Ara} , MCS 2, <i>cat</i> , ori pBR322	(9)
pBAD33	P _{Ara} , MCS 2, <i>cat</i> , ori p15A	(9)
pBGG59	Fusion of <i>E.c. glmZ</i> ' (-424 to +32) to <i>lacZ</i>	(10)
pBGG111	Fusion of E.c. glmZ' (-207 to +32) to lacZ	this work
pBGG112	Fusion of <i>E.c. glmZ</i> ' (-100 to +32) to <i>lacZ</i>	this work
pBGG113	Fusion of <i>E.c. glmZ</i> (-80 to +32) to <i>lacZ</i>	this work
pBGG114	Fusion of <i>E.c. glmZ</i> (-40 to +32) to <i>lacZ</i>	this work
pBGG135	Fusion of <i>E.c. glmZ</i> ' (-11 to +32) to <i>lacZ</i>	this work
pBGG157	Fusion of <i>E.c. glmZ</i> (-40 to +32) to <i>lacZ</i> , -35 region mutated	this work
pBGG170	Fusion of <i>E.c. glmZ</i> ' (-20 to +32) to <i>lacZ</i>	this work
pBGG171	Fusion of <i>E.c. glmZ</i> (-40 to +32) to <i>lacZ</i> , -10 region mutated	this work
pBGG201	Fusion of <i>E.c. glmY</i> ' (-238 to +22) to <i>lacZ</i>	(11)
pBGG209	Fusion of E.c. glmY (-238 to +22) to lacZ, -10 region mutated	(11)
pBGG219	<i>E.c. glrR</i> :: His ₁₀ in pKES170	(11)
pBGG223	E.c. glrR under P _{Ara} -control in pBAD18-cm	(11)
pBGG389	<i>E.c. glrR</i> under <i>P</i> _{Ara} -control in pBAD33	this work
pBGG390	Fusion of E.c. glmY' (-238 to +22) to lacZ, IHF1 mutated	this work
pBGG391	Fusion of E.c. glmY' (-238 to +22) to lacZ, -10 region and IHF1 mutated	this work
pBGG397	Y.p. glrR:: His ₁₀ in pKES170	this work
pBGG398	<i>E.c. glrR</i> (D56A) under P_{Ara} -control in pBAD33	this work
pBGG399	<i>E.c. glrR</i> (D56E) under P_{Ara} -control in pBAD33	this work
pKEM04	Promoter-less <i>lacZ, kan, attP, aadA,</i> ori p15A	(12)
pKES15	bgi'-lacZ, kan, attP, aadA, ori p15A	(12)
pKES170	lacl ⁻ , Ptac, T7gene10-RBS, Ndel, Xbal, rrnBT1/T2, bla,pBR322-ori	(11)
pLDR8	λ int under control of λP_R , λcl_{857} , kan, ori pSC101-rep ¹⁵	(13)
pYG1	Fusion of Y.p. glmY' (-257 to +22) to lacZ	this work
pYG2	Fusion of Y.p. glmZ' (-303 to +22) to lacZ	this work
pYG6	Y.p. glrR under P_{Ara} -control in pBAD18-cm	this work
pYG7	Fusion of S.t. glmY' (-242 to +22) to lacZ	this work
pYG8	Fusion of S.t. gImZ' (-242 to +22) to lacZ	this work
pYG9	Fusion of Y.p. glmZ' (-292 to +22) to lacZ, ABS1 mutated	this work
pYG10	Fusion of Y.p. glmZ' (-303 to +22) to lacZ, ABS2 mutated	this work
pYG11	Fusion of Y.p. glmZ' (-303 to +22) to lacZ, ABS3 mutated	this work
pYG12	Fusion of Y.p. glmZ' (-292 to +22) to lacZ, ABS1,2,3 mutated	this work

TABLE S1. Plasmids used in this study

^aPositions are relative to the first nucleotide of the respective gene. Gene names are according to http://ecocyc.org/.

TABLE S2. Oligonucleotides used in this study

Primer		Rec	Position ^D
FIIIIEI	di Sequence Res.		FOSICION
BC200	CCACCCCTCCACCATCCTCTTTTACTTTTACCCCC	Sall	$E_{c} alm 7_{-}207 \text{ to }_{-}183$
BC200		Yhal	E_{c} $dmZ + 32$ to +6
BG202		Soll	$E_{c} a m 7 100 \text{ to } 76$
BG333		Sall	$E_{10} = 100 \text{ to } 1000 \text{ to } 1000 \text{ to } 100 \text{ to } 1000 \text{ to } 1000 \text{ to } 100$
DG334		Sall	E.C.GIIIIZ -60 [0 -55]
BG335		Sali	E.C.GIIIIZ -40 [0 - 17]
BG347	TCGCCT		E.C.gimz -11 to +32
BG348	[P]-CTAGAGGCGAACATAAGAGATGGAATGAGCATCTACTCGTT TATTATG		<i>E.c.glmZ</i> +32 to -11
BG377	GCACGCGTCGACCTTTTTTGTGTCTGTAAATCACG	Sall	E c almY -238 to -213
BG388	GCACGCGTCGACAGGGA AA TT T TTTCCCGATTCTCTGTG	Sall	E c alm 7 - 40 to -13
BG411	GCACGCGTCGACCTCTGTGGCATAATAAACGAG	Sall	E c alm Z - 20 to -1
BG412	GCACGCGTCGACAGGGATGTTATTTCCCCGATTCTCTGTGGCATG	CG Sall	E c alm Z -40 to +10
DOTIE	AAACGAGTAGATGCTC	e cui	2.0.gim2 10 to 10
BG456	GCTCTAGAATAAGTCGGTGAATGAGCCAC	Xbal	E.c. glmY +22 to +2
BG490	GCGAGCTCCCATCCACCCATGAGGTCAC	Sacl	E.c. glrR -25 to -5
BG491	GGCTCTAGATCATTCCTTGAAATCGTTTGCATC	Xbal	<i>E.c. glrR</i> +1335 to +1311
BG578	CGGTGAAGGGCAATCAGCTG		E.c. lacZ -271 to -252
BG579	GGCCTCTTCGCTATTACGCC		<i>E.c. lacZ</i> +129 to +110
BG580	ATTAATGCAGCTGGCACGACAG		<i>E.c. lacZ</i> -171 to -150
BG581	ACGGCCAGTGAATCCGTAATC		<i>E.c. lacZ</i> +29 to + 9
BG684	[P]-GCGACACTTAACTCACCCCTTTTAATATTATCTAATAAGTTTATC	0	<i>E.c. glmY</i> -185 to -141
BG685	PJ-GTAGATTTAGTCATCAGC GCT CTGCGGATGGATGAAATG		<i>E.c. glrR</i> +148 to +186
BG686	P-GTAGATTTAGTCATCAGC GAA CTGCGGATGGATGAAATG		<i>E.c. glrR</i> +148 to +186
BG696	CTCGTACTCATATGACACCACGCAAACC	Ndel	Y.p. glrR +1 to +17
BG697	CGTCTCTAGACTCTTTAAAATCGTTGGCATCC	Xbal	Y.p. glrR +1335 to +1314
BG698	GCACGCGTCGACTTTTTATATTCTGTCGGCAAG	Sall	Y.p. glmY -257 to -236
BG699	CGTCTCTAGACATAAAAAGGTGAATGAGCAAC	Xbal	Y.p. glmY +22 to +1
BG700	GCACGCGTCGACTTCGTTGTGTTGGGCGTCAG	Sall	Y.p. glmZ -303 to -284
BG701	CGTCTCTAGAAATAAGTGGGATGAGCATCTAC	Xbal	Y.p. glmZ +22 to +1
BG727	GCGAGCTCAAGGAATCTCATGACACCACG	Sacl	Y.p. glrR -10 to + 11
BG728	GGCTCTAGATTACTCTTTAAAATCGTTGGCATC	Xbal	Y.p. glrR +1338 to + 1315
BG747	GCACGC <u>GTCGAC</u> GGGCGTCAG ACA TGGTTTTCCACGACAATAAA	C Sall	Y.p. glmZ -292 to -259
	G		
BG748	[P]-TGTCACCTTCTCACG TGT ATGTGATCGTTT		Y.p. glmZ -234 to -263
BG750	GCACGC <u>GTCGAC</u> CAAGATTAAAGTGTCGGGAAATCC	Sall	S.t. glmY -242 to -219
BG751	CGTC <u>TCTAGA</u> CATAAGAAGGTGAATGAGCCAC	Xbal	S.t. glmY +22 to +1
BG752	GCACGC <u>GTCGAC</u> GTGTTGCCATTATGATTTGTTGG	Sall	S.t. glmZ -242 to -219
BG753	CGTC <u>TCTAGA</u> TAAGAGATGGAATGAGCATCTAC	Xbal	S.t. glmZ +22 to +1
BG754	[P]- CAATGTAGGGTTATAA ACAA GTTTTGTAGCGACAG		Y.p. glmZ -204 to -170
BG755	GTTCACTCTGGTCACCGGG		Y.p. glmZ -170 to -152

^aRestriction sites are underlined; Nucleotide positions that differ from the wild-type sequence are in boldface; [P] indicates 5'-phosphorylation of the oligonucleotide. ^bPositions are relative to the first nucleotide of the respective gene. Gene names are according to http://ecocyc.org/.

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