Supplementary material for:

Common and divergent features in transcriptional control of the homologous small RNAs GlmY and GlmZ in *Enterobacteriaceae*

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Running title: Regulation of sRNA GlmY and GlmZ expression

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Supplemental "Materials and Methods"

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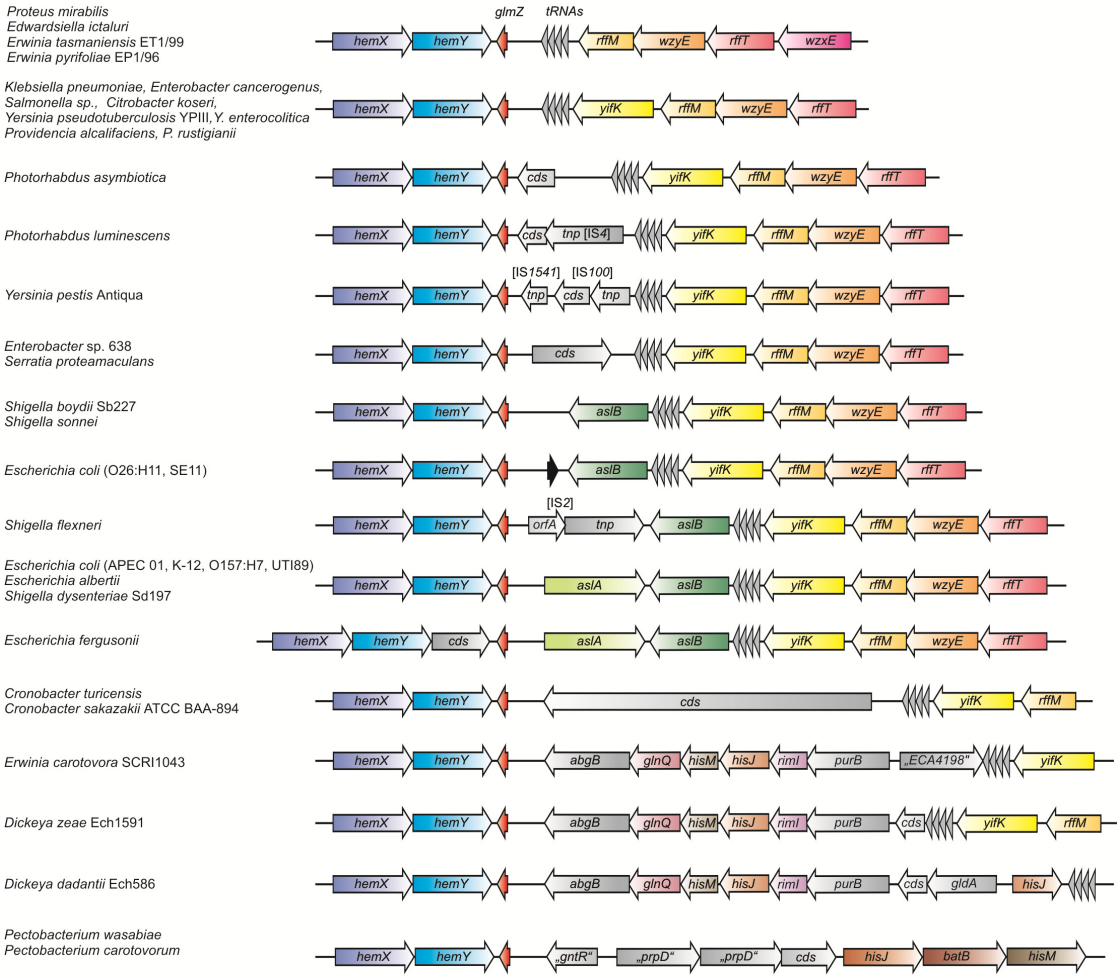
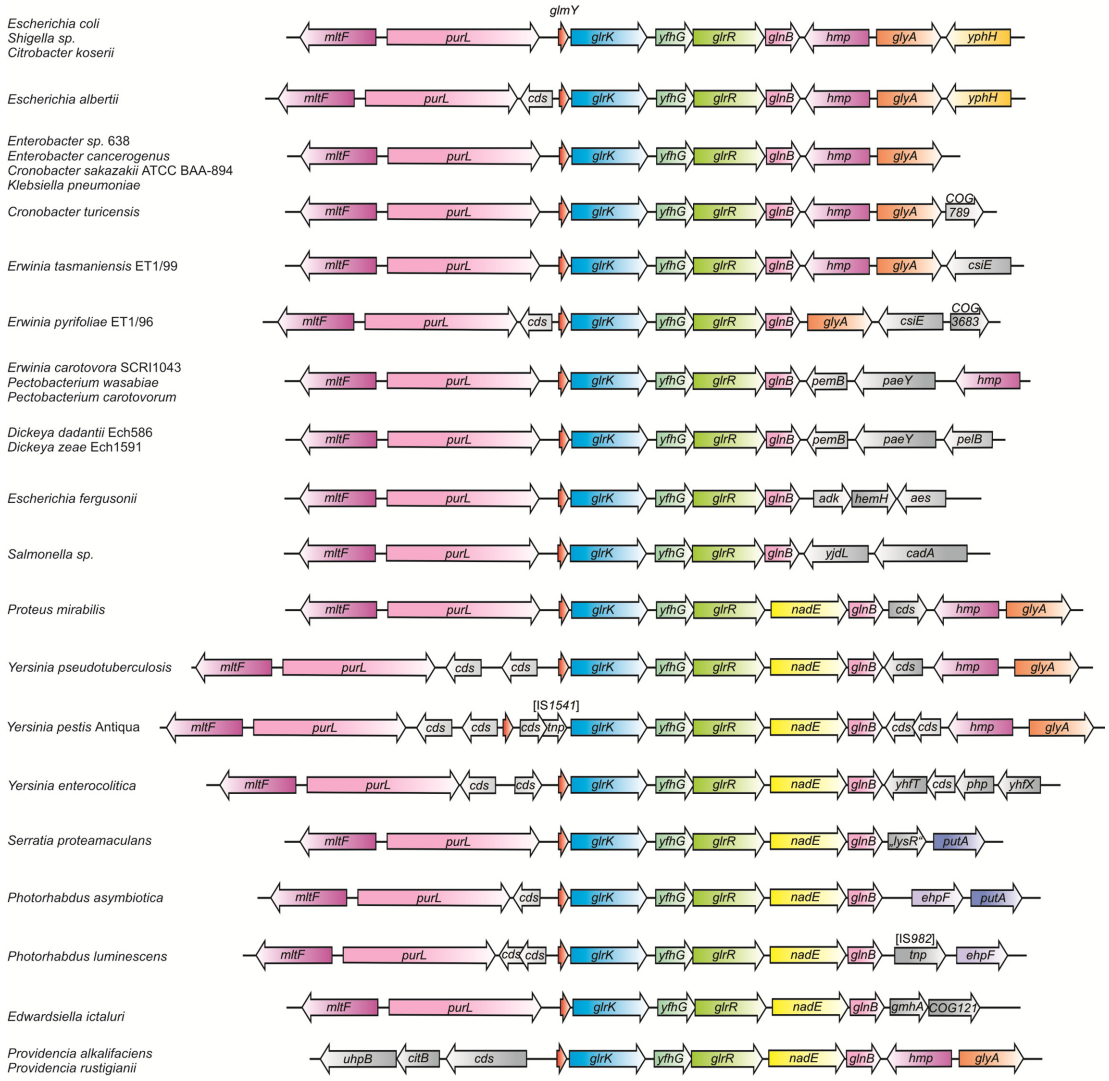
Fig. S1



abgB : putative peptidase
araC : AraC-type transcriptional regulator
aslA : arylsulfatase
aslB : sulfatase maturation enzyme
batB : amino acid ABC transporter, membrane subunit
cds : hypothetical protein
COG4313 : protein involved in phenol degradation
COG4667 : predicted esterase of the alpha-beta hydrolase superfamily
ECA4198 : putative exported phosphatase
gldA : putative glycerol dehydrogenase
glnQ : putative amino-acid ABC transporter ATP-binding protein
gntR : GntR-type transcriptional regulator
hisJ : putative amino-acid ABC transporter binding protein
hisM : probable amino-acid ABC transporter permease protein
holD : DNA polymerase III, psi subunit
lysR : LysR-type transcriptional regulator
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-acetyl-D-mannosaminuronic acid transferase
rffT = *wecF* : Fuc4NAc (4-acetamido-4,6-dideoxy-D-galactose) transferase
rimI : putative acetyltransferase
rsmC : 16S RNA G1207 methylase
tas : predicted oxidoreductase (related to aryl-alcohol dehydrogenases)
tatD : Mg-dependent DNase
tnp : transposase
wzxE : O-antigen translocase (flippase)
wzyE = *rffT* = *wecF* : subunit of common antigen biosynthesis complex
yifK : amino 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.

Fig. S2



adk : adenylate kinase
aes : acetylesterase
cadA : lysine decarboxylase
cds : hypothetical protein
citB : response regulator with CheY-like receiver domain
COG121 : predicted glutamine amidotransferase
COG789 : SoxR-type transcriptional regulator
COG3683 : ABC-type transport system, periplasmic component
csiE : stationary phase-inducible protein
ehpF : putative AMP-dependent synthetase/ligase
glnB : protein PII
glyA : serine hydroxymethyltransferase
gmhA : putative phosphoheptose isomerase
hemH : ferrochelatase
hmp : flavohemoglobin
lysR : LysR-type transcriptional regulator
mltF = yfhD : membrane-bound lytic murein transglycosylase F
nadE : glutamine-dependent NAD(+) synthetase
paeY : pectin acetylesterase
pelB : pectate lyase
pemB : pectinesterase
php : predicted metal-dependent hydrolase
purL : phosphoribosylformylglycinamide synthetase
putA : aldehyde dehydrogenase
tnp : transposase
uhpB : histidine kinase
yhfT : putative integral membrane protein
yhfX : predicted amino acid racemase
yjdL : peptide:H^+ symporter
yphA : DoxX family protein
yphH : NagC-like 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.

Fig. S3

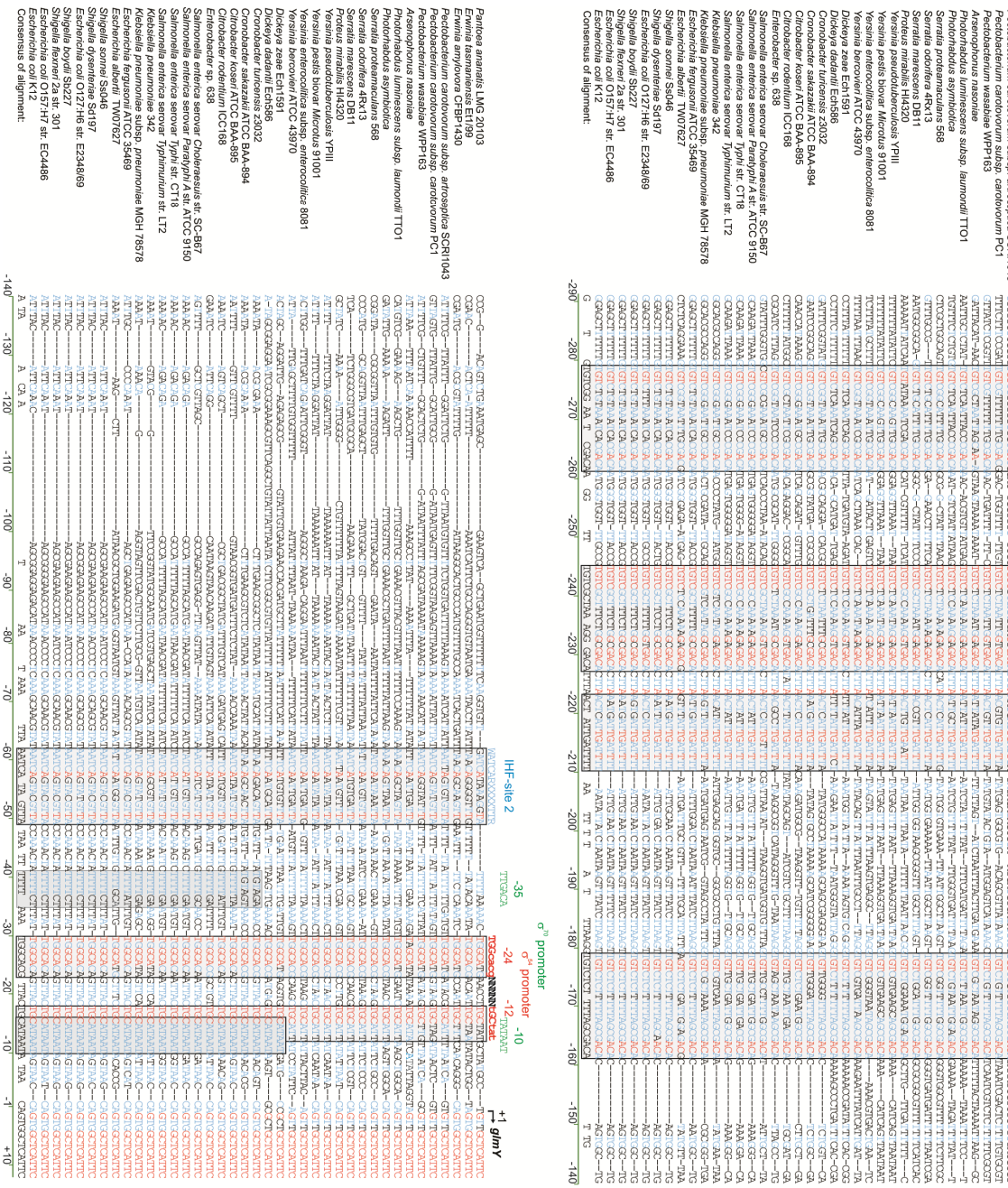


Fig. S3. Sequence alignment of the *glmY* promoter regions from 39 enterobacterial genomes. Fully conserved nucleotide positions are highlighted in red, while residues conserved in the majority of these sequences are in blue. The transcriptional start site of *glmY* is marked with an arrow. The GlrR 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

(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 proteamaculans* 568 (NC_009832.1), *Serratia odorifera* 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 pesti*s 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^{TM} 9.0.

Fig. S4

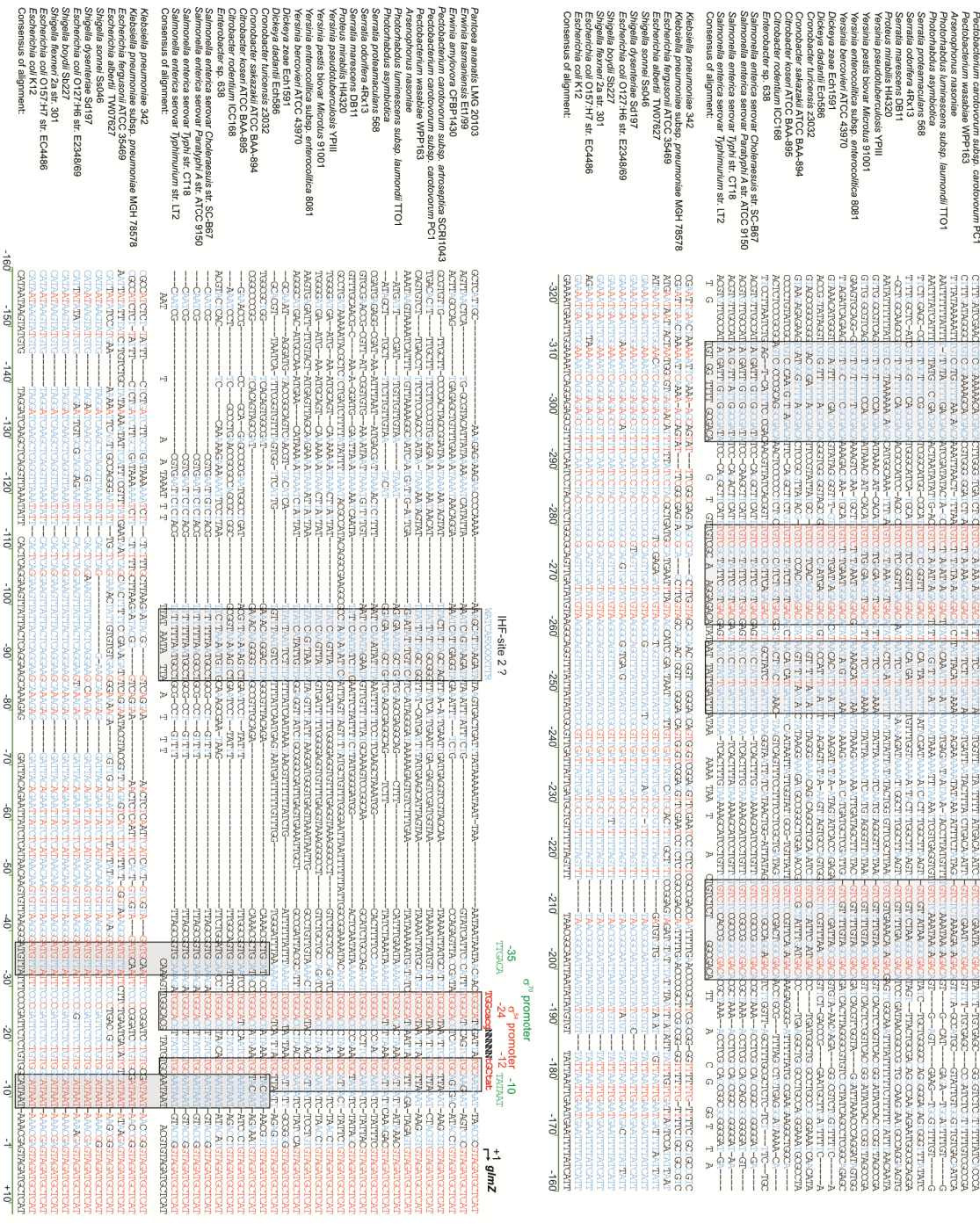


Fig. S4. Sequence alignment of the *glmZ* promoter regions from 39 enterobacterial genomes. The sequences classified into two groups, which exhibited no significant homologies to each other and are therefore shown in separate alignments. Fully conserved nucleotide positions within each group are highlighted 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

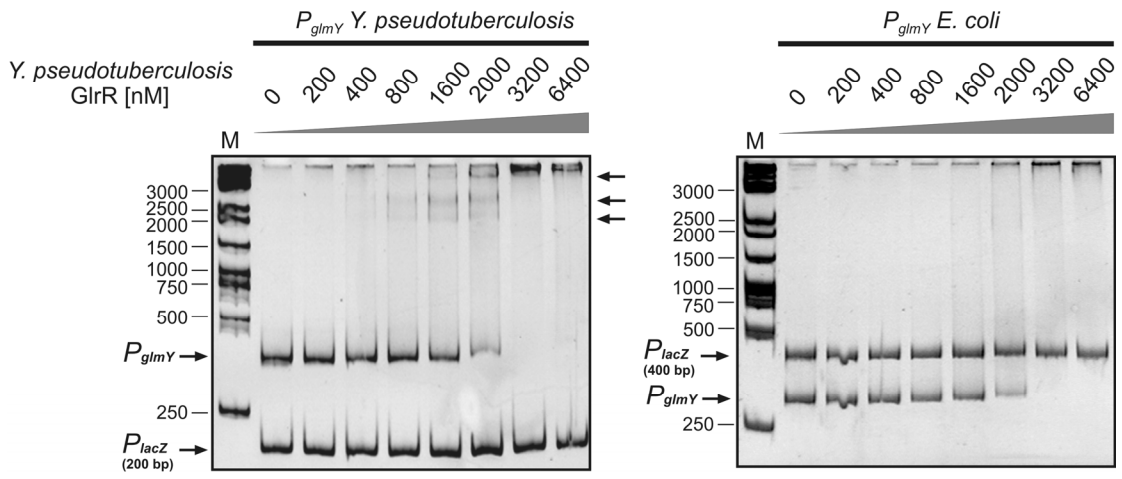


Fig. S5. *Y. pseudotuberculosis* GlrR binds the *glmY* promoters of *Y. pseudotuberculosis* and *E. coli*. EMSAs to test binding of *Y. pseudotuberculosis* GlrR protein to the *glmY* promoter regions of *Y. pseudotuberculosis* (-257 to +22) and *E. coli* (-238 to +22). The DNA fragments were obtained 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

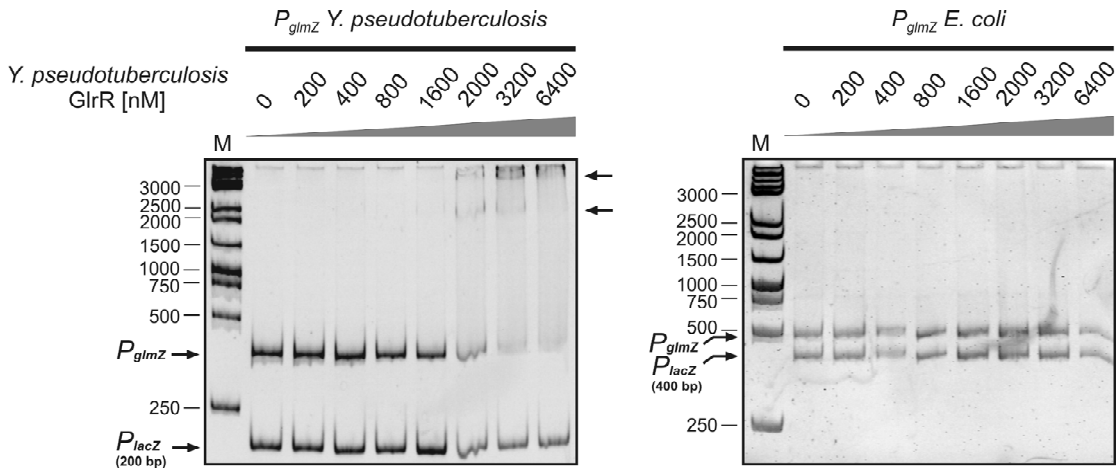


Fig. S6. *Y. pseudotuberculosis* GlrR binds the cognate *glmZ* promoter, while the *E. coli glmZ* promoter is not bound. EMSAs to test binding of *Y. pseudotuberculosis* GlrR protein to the *glmZ* promoter regions of *Y. pseudotuberculosis* (-303 to +22) and *E. coli* (-424 to +32). The DNA fragments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

Fig. S7

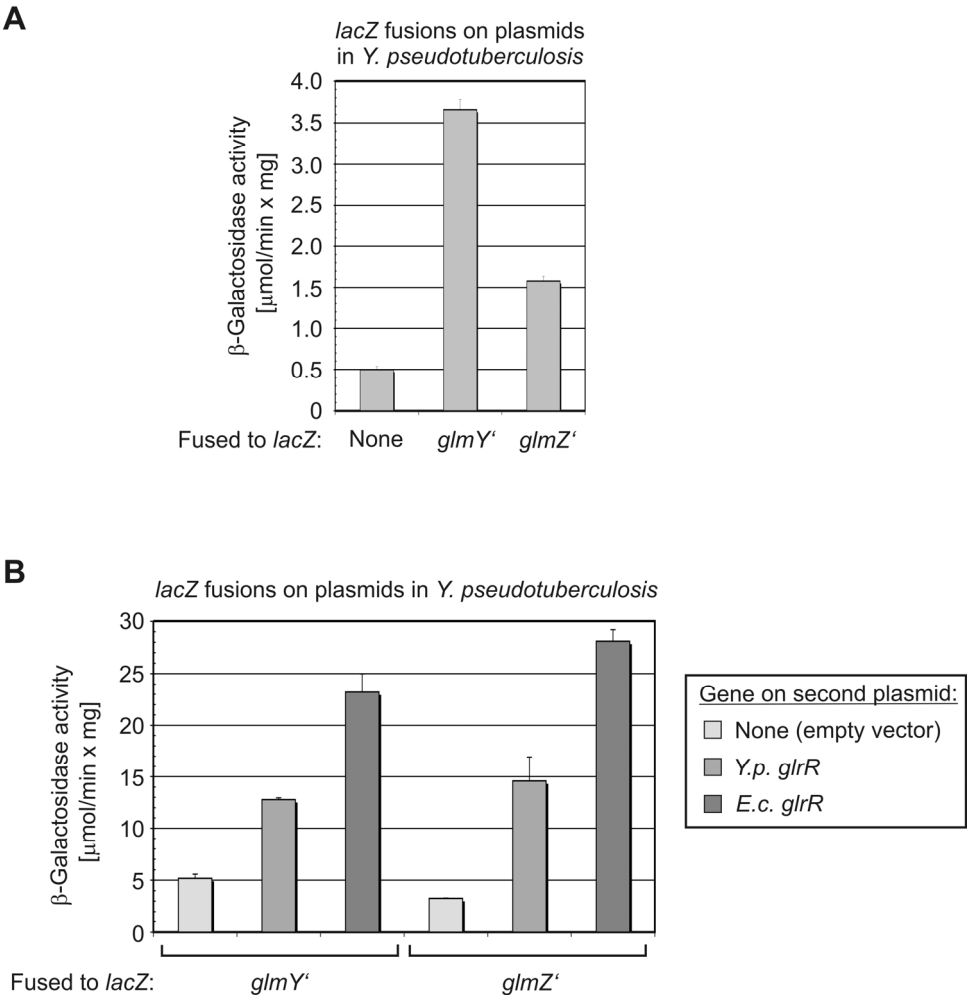


Fig. S7. Expression of *glmY* and *glmZ* in *Y. pseudotuberculosis*. (**A**) *glmY* and *glmZ* are expressed in *Y. pseudotuberculosis*. β-Galactosidase activities of *Y. pseudotuberculosis* carrying a *glmY'-lacZ* (column 2) or *glmZ'-lacZ* fusion (column 3) on a plasmid (pYG1 and pYG2, respectively). Cells carrying the empty *lacZ* fusion vector pKEM04 served as background control (column 1). (**B**) Stimulation of *glmY* and *glmZ* expression by GlrR in *Y. pseudotuberculosis*. Additional plasmids carrying either *glrR* from *Y. 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 P_{Ara} 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 β-galactosidase activities were determined.

Fig. S8

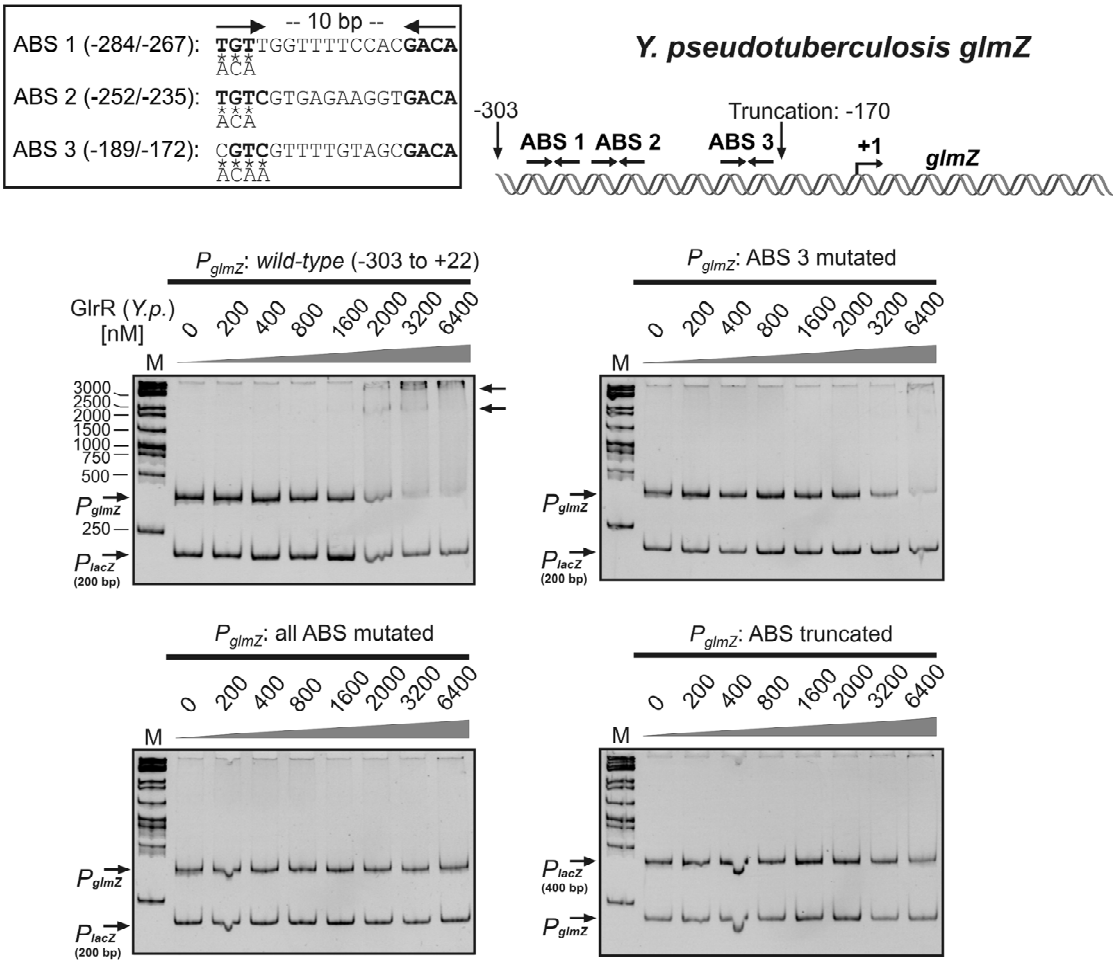


Fig. S8. Binding of the *Y. pseudotuberculosis* GlrR protein to its cognate *glmZ* promoter requires three activator binding sites. EMSAs to monitor binding of *Y. pseudotuberculosis* GlrR to DNA fragments covering the *Y. pseudotuberculosis glmZ* promoter (-303 to +22). DNA fragments were tested that 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

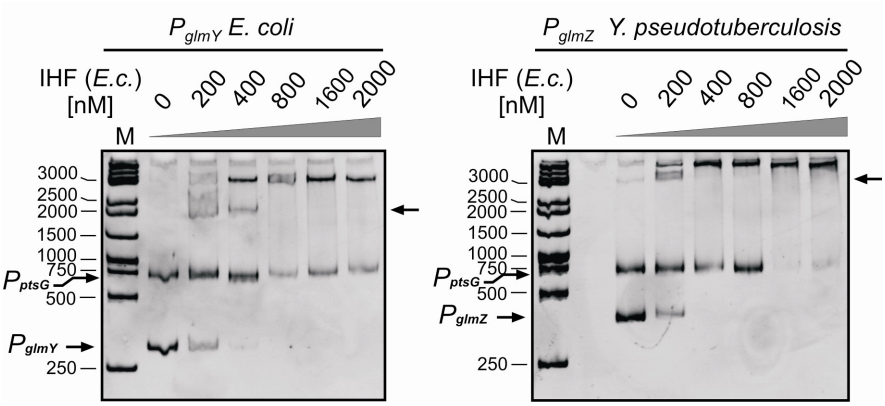


Fig. S9. Binding of IHF to the *E. coli glmY* and the *Y. pseudotuberculosis glmZ* promoter regions as revealed by EMSA. The DNA fragments were obtained by PCR making use of the primer pairs BG377/BG456 and BG700/BG701, respectively. As a difference to the experiments shown in Fig. 6 B, a DNA fragment 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).

Fig. S10

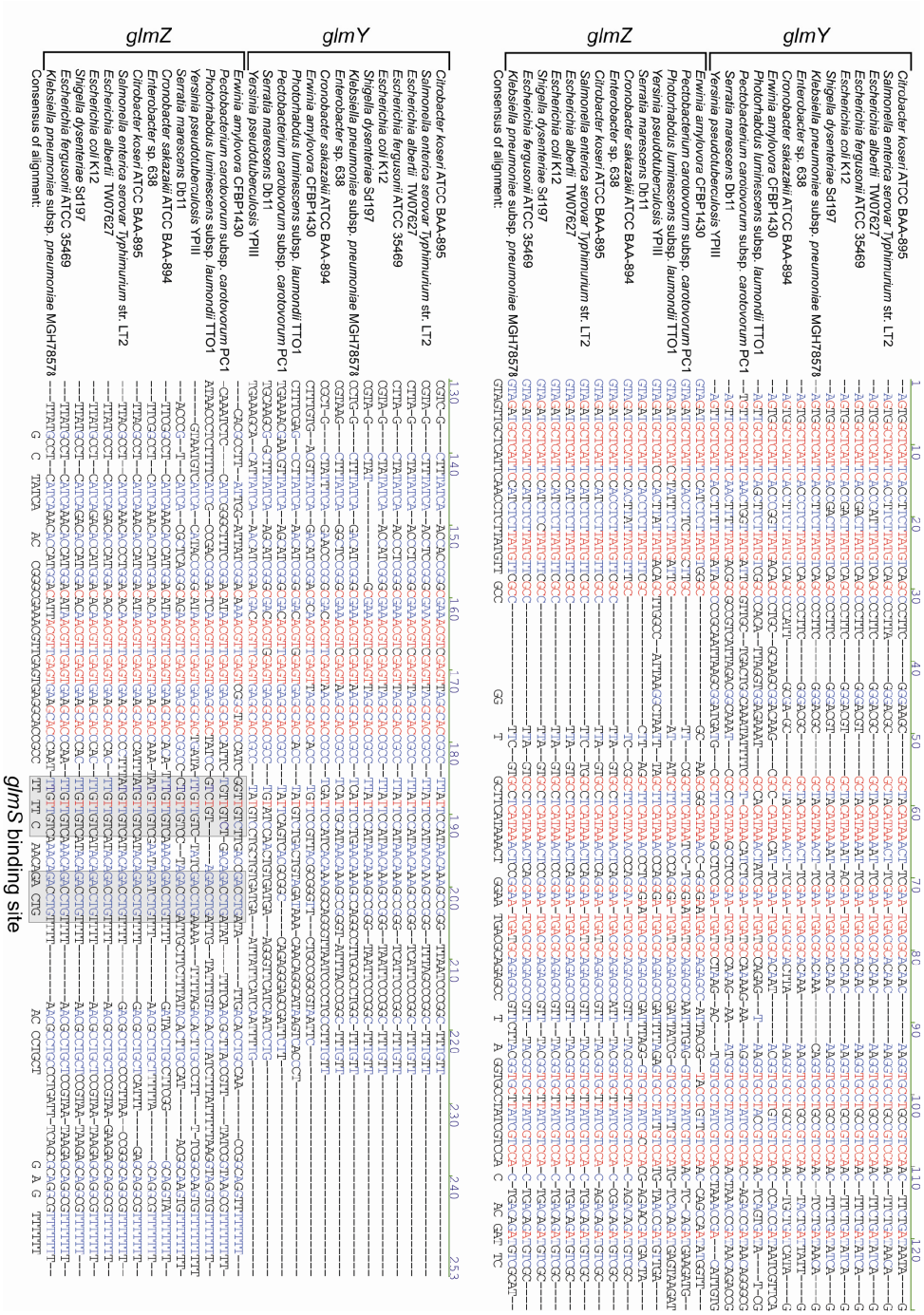


Fig. S10. Sequence alignment of *glmY* and *glmZ* genes of 14 enterobacterial species. Fully conserved nucleotide positions are highlighted in red, while residues conserved in the majority of sequences are in blue. The positions involved in GlmZ/*glmS* interaction (according to (5,6)) are boxed. Refer 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 Advance™ 9.0.

Fig. S11

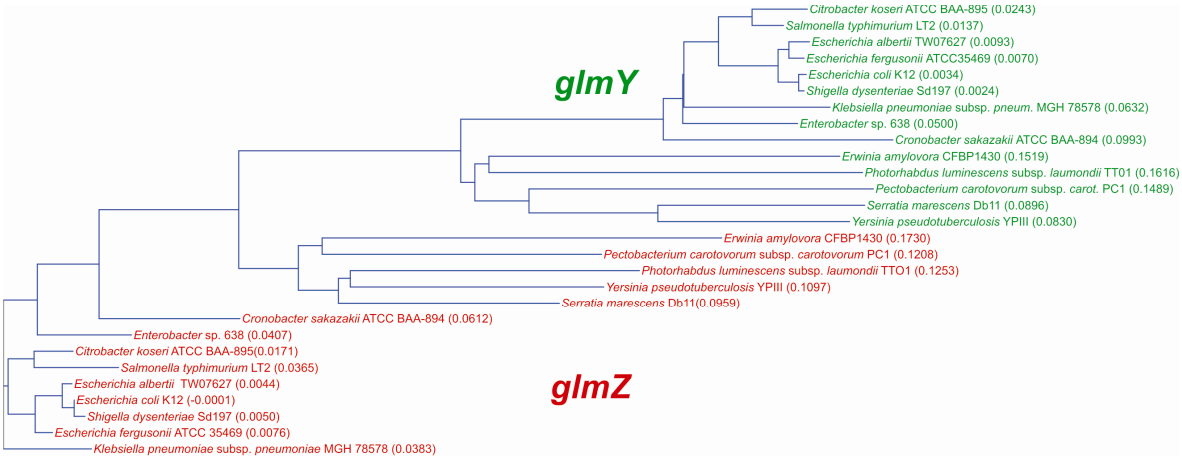


Fig. S11. Phylogenetic tree of *glmY* and *glmZ* genes of 14 enterobacterial species. The tree was calculated from the sequence alignment shown in Fig. S10 using the AlignX tool of software Vector NTI Advance^{TM} 9.0. The tree is built using the Neighbor joining method (7), which works on a matrix of 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

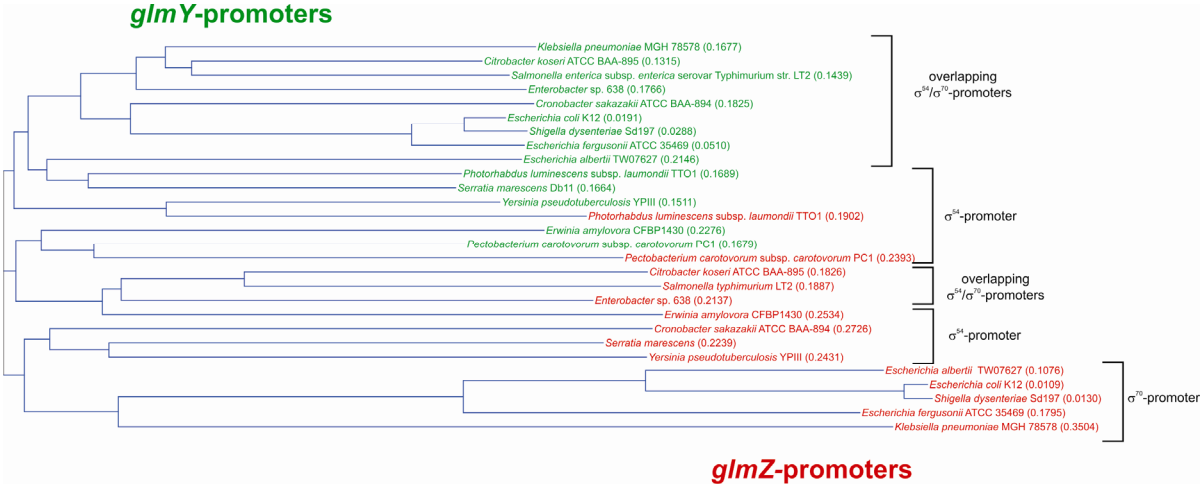


Fig. S12. Phylogenetic tree of *glmY*- and *glmZ*-promoter regions of 14 enterobacterial species. The tree was calculated from a sequence alignment (data not shown) comprising the promoter sequences of the *glmY*- and *glmZ*-genes used for Fig. S11. The used sequences are shown in Figures S3 and S4, 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 *glmZ* (-303 to +22) to *lacZ*, the corresponding *glmY*-5' and *glmZ*-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 SalI-XbaI 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 SalI/XbaI-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 SalI/XbaI-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 SalI/XbaI 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 SalI/XbaI into plasmid pKES15. To introduce the mutation in the -10 sequence, the AflII-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 SalI/XbaI 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 *glmZ'*(-11 to +32)-*lacZ* fusion was constructed by ligation of hybridized 5'-phosphorylated oligonucleotides BG347 and BG348 with the SalI/XbaI-digested 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 SalI/XbaI 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 NdeI- and XbaI-sites on plasmid pKES170. For construction of plasmid pYG6 carrying *Y. pseudotuberculosis glrR* under P_{Ara} promoter control, *glrR* was amplified using primers BG727/BG728 and cloned between the SacI and XbaI sites on plasmid pBAD18-cm. To construct plasmid pBGG389, which carries *E. coli glrR* under P_{Ara} control, the SacI-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 SacI/XbaI sites on plasmid pBAD33.

SUPPLEMENTAL TABLES

TABLE S1. Plasmids used in this study

Name	Genotype or relevant structures[a]	Reference or construction
pBAD18-cm	P_{Ara}, MCS 2, *cat*, ori pBR322	(9)
pBAD33	P_{Ara}, MCS 2, *cat*, ori p15A	(9)
pBGG59	Fusion of *E.c. glmZ*' (-424 to +32) to *lacZ*	(10)
pBGG111	Fusion of *E.c. glmZ*' (-207 to +32) to *lacZ*	this work
pBGG112	Fusion of *E.c. glmZ*' (-100 to +32) to *lacZ*	this work
pBGG113	Fusion of *E.c. glmZ* (-80 to +32) to *lacZ*	this work
pBGG114	Fusion of *E.c. glmZ* (-40 to +32) to *lacZ*	this work
pBGG135	Fusion of *E.c. glmZ'* (-11 to +32) to *lacZ*	this work
pBGG157	Fusion of *E.c. glmZ* (-40 to +32) to *lacZ*, -35 region mutated	this work
pBGG170	Fusion of *E.c. glmZ*' (-20 to +32) to *lacZ*	this work
pBGG171	Fusion of *E.c. glmZ* (-40 to +32) to *lacZ*, -10 region mutated	this work
pBGG201	Fusion of *E.c. glmY'* (-238 to +22) to *lacZ*	(11)
pBGG209	Fusion of *E.c. glmY*' (-238 to +22) to *lacZ,* -10 region mutated	(11)
pBGG219	*E.c. glrR*:: His_{10} in pKES170	(11)
pBGG223	*E.c. glrR* under P_{Ara}-control in pBAD18-cm	(11)
pBGG389	*E.c. glrR* under P_{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	*E.c. glrR* (D56A) under P_{Ara}-control in pBAD33	this work
pBGG399	*E.c. glrR* (D56E) under P_{Ara}-control in pBAD33	this work
pKEM04	Promoter-less *lacZ, kan, attP, aadA,* ori p15A	(12)
pKES15	*bgl'-lacZ, kan, attP, aadA,* ori p15A	(12)
pKES170	$lacI^q$, *Ptac*, T7gene10-RBS, NdeI, XbaI, *rrnBT1/T2*, *bla*,pBR322-ori	(11)
pLDR8	λ *int* under control of λP_R, λcI_{857}, *kan*, ori pSC101-rep^{TS}	(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. glmZ'* (-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

[a]Positions 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	Sequence[a]	Res. Sites	Position[b]
BG200	GCACGCGTCGACGATGCTGTTTTTAGTTTTAACGGC	SalI	*E.c. glmZ* -207 to -183
BG202	GCGTCTAGAGGCGAACATAAGAGATGGAATGAGC	XbaI	*E.c. glmZ* +32 to +6
BG333	GCACGCGTCGACTCAGGAAGTTATTACTCAGGAAG	SalI	*E.c.glmZ* -100 to -76
BG334	GCACGCGTCGACAAGCAAAGAGGATTACAGAATTATC	SalI	*E.c.glmZ* -80 to -55
BG335	GCACGCGTCGACAGGGATGTTATTTCCCGATTCTC	SalI	*E.c.glmZ* -40 to -17
BG347	[P]-TCGACATAATAAACGAGTAGATGCTCATTCCATCTCTATTGT TCGCCT		*E.c.glmZ* -11 to +32
BG348	[P]-CTAGAGGCGAACATAAGAGATGGAATGAGCATCTACTCGTT TATTATG		*E.c.glmZ* +32 to -11
BG377	GCACGCGTCGACCTTTTTTGTGTCTGTAAATCACG	SalI	*E.c. glmY* -238 to -213
BG388	GCACGCGTCGACAGGGA**AA**TT**T**TTTCCCGATTCTCTGTG	SalI	*E.c.glmZ* -40 to -13
BG411	GCACGCGTCGACCTCTGTGGCATAATAAACGAG	SalI	*E.c.glmZ* -20 to -1
BG412	GCACGCGTCGACAGGGATGTTATTTCCCGATTCTCTGTGGCAT**GCG** AAACGAGTAGATGCTC	SalI	*E.c.glmZ* -40 to +10
BG456	GCTCTAGAATAAGTCGGTGAATGAGCCAC	XbaI	*E.c. glmY* +22 to +2
BG490	GCGAGCTCCCATCCACCCATGAGGTCAC	SacI	*E.c. glrR* -25 to -5
BG491	GGCTCTAGATCATTCCTTGAAATCGTTTGCATC	XbaI	*E.c. glrR* +1335 to +1311
BG578	CGGTGAAGGGCAATCAGCTG		*E.c. lacZ* -271 to -252
BG579	GGCCTCTTCGCTATTACGCC		*E.c. lacZ* +129 to +110
BG580	ATTAATGCAGCTGGCACGACAG		*E.c. lacZ* -171 to -150
BG581	ACGGCCAGTGAATCCGTAATC		*E.c. lacZ* +29 to + 9
BG684	[P]-GCGACACTTAACTCA**CCCC**TTTTAATATTATCTAATAAGTTTATC		*E.c. glmY* -185 to -141
BG685	[P]-GTAGATTTAGTCATCAGC**GCT**CTGCGGATGGATGAAATG		*E.c. glrR* +148 to +186
BG686	[P]-GTAGATTTAGTCATCAGC**GAA**CTGCGGATGGATGAAATG		*E.c. glrR* +148 to +186
BG696	CTCGTACTCATATGACACCACGCAAACC	NdeI	*Y.p. glrR* +1 to +17
BG697	CGTCTCTAGACTCTTTAAAATCGTTGGCATCC	XbaI	*Y.p. glrR* +1335 to +1314
BG698	GCACGCGTCGACTTTTTTATATTCTGTCGGCAAG	SalI	*Y.p. glmY* -257 to -236
BG699	CGTCTCTAGACATAAAAAGGTGAATGAGCAAC	XbaI	*Y.p. glmY* +22 to +1
BG700	GCACGCGTCGACTTCGTTGTGTTGGGCGTCAG	SalI	*Y.p. glmZ* -303 to -284
BG701	CGTCTCTAGAAATAAGTGGGATGAGCATCTAC	XbaI	*Y.p. glmZ* +22 to +1
BG727	GCGAGCTCAAGGAATCTCATGACACCACG	SacI	*Y.p. glrR* -10 to + 11
BG728	GGCTCTAGATTACTCTTTAAAATCGTTGGCATC	XbaI	*Y.p. glrR* +1338 to + 1315
BG747	GCACGCGTCGACGGGCGTCAG**ACA**TGGTTTTCCACGACAATAAAC G	SalI	*Y.p. glmZ* -292 to -259
BG748	[P]-TGTCACCTTCTCACG**TGT**ATGTGATCGTTT		*Y.p. glmZ* -234 to -263
BG750	GCACGCGTCGACCAAGATTAAAGTGTCGGGAAATCC	SalI	*S.t. glmY* -242 to -219
BG751	CGTCTCTAGACATAAGAAGGTGAATGAGCCAC	XbaI	*S.t. glmY* +22 to +1
BG752	GCACGCGTCGACGTGTTGCCATTATGATTTGTTGG	SalI	*S.t. glmZ* -242 to -219
BG753	CGTCTCTAGATAAGAGATGGAATGAGCATCTAC	XbaI	*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

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

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