

Figure S1

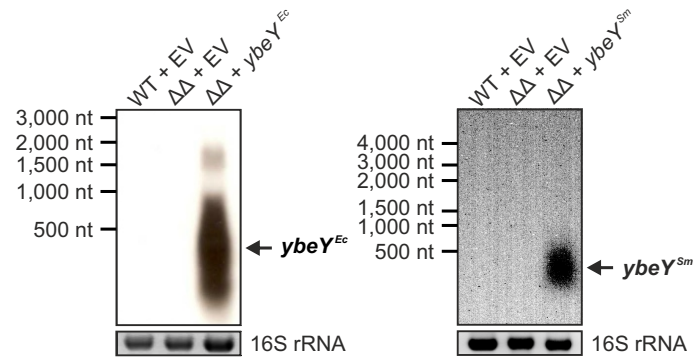


Figure S1. Northern blot of RNA isolated from a $\Delta hfq \Delta ybeY$ strain, complemented with plasmid-encoded *ybeY^{Ec}* (*E. coli*, left) or *ybeYSm* (*S. meliloti*, right).

Figure S2

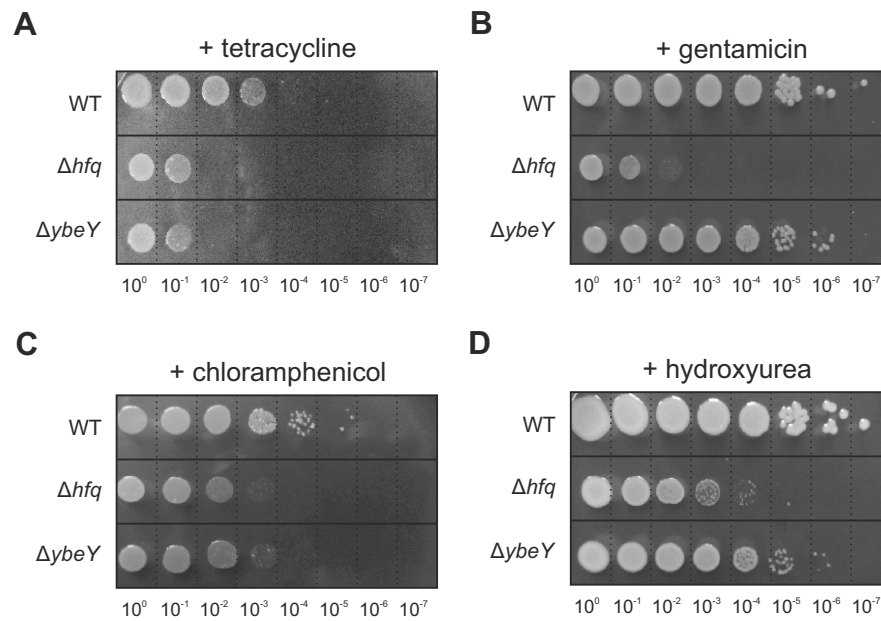
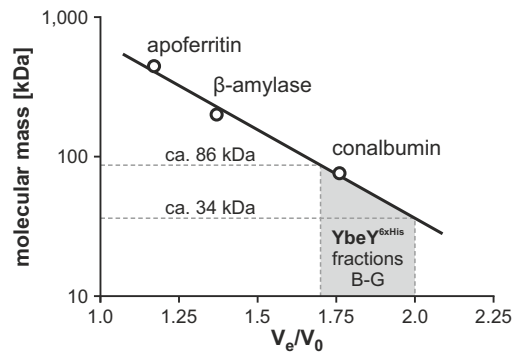


Figure S2. Application of 1 $\mu\text{g/ml}$ tetracycline (A), 4 $\mu\text{g/ml}$ gentamicin (B), 15 $\mu\text{g}/\mu\text{l}$ chloramphenicol (C) or 20 mM hydroxyurea (D) was used to assess stress tolerance of Δhfq and $\Delta ybeY$ strains. The ribosome targeting antibiotics chloramphenicol and tetracycline had the strongest effect on the *ybeY* deletion strain but also inhibited growth of the *hfq* mutant. Gentamicin did not affect growth of the *ybeY* mutant, but drastically impaired growth of the *hfq* mutant. Addition of 20 mM hydroxyurea led to slightly reduced growth of Δhfq and $\Delta ybeY$ strains.

Figure S3

A



B

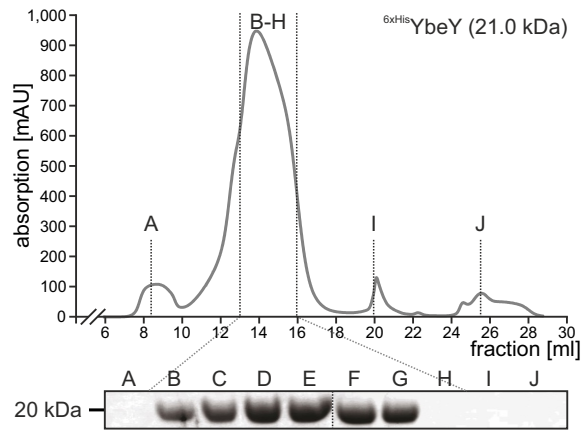


Figure S3. Gel filtration chromatography of standard proteins and ^{6xHis}YbeY. (A) Blue dextran (2,000 kDa) was used to determine the void volume (V_0). Apoferritin (443 kDa), β -amylase (200 kDa) and conalbumin (76 kDa) were measured during GPC to calculate a standard curve based on the maximal absorption of the corresponding proteins and their relative molecular mass. (B) GPC of ^{6xHis}YbeY was done as in Fig. 8.

Figure S4

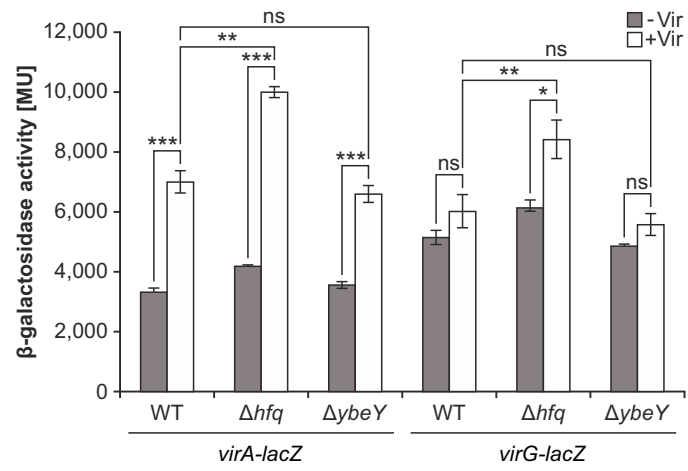


Figure S4. *virA-lacZ* and *virG-lacZ* β -galactosidase activity in *A. tumefaciens* WT, Δhfq and $\Delta ybeY$ strains. *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns, non-significant.

Table S1. Proteins differentially abundant in *A. tumefaciens* $\Delta ybeY$ (iTRAQ)

up-regulated proteins (FC>1.35)

Accession	Annotation	Protein	Description	KEGG ontology (1)	$\Delta ybeY/WT$ ratio (2)	$\Delta ybeY/WT$ variability [%]	$\Delta hfq/WT$ ratio	replicates
AAK88993	Atu4447	-	ABC transporter, substrate binding protein (sorbitol)	[1]	1.993	8.5	0.42	3
AAK89316	Atu4115	-	ABC transporter, membrane spanning protein (dipeptide)	[1]	1.910	27.6	-	2
AAK89532	Atu3887	HspL	small heat shock protein	[5]	1.865	1.6	-	3
AAK86885	Atu1076	CvpA	colicin V production protein	[7]	1.644	22.3	-	2
AAK86357	Atu0545	FlaA	flagella associated protein	[4]	1.631	24.3	-	3
AAK90221	Atu3165	-	ABC transporter, substrate binding protein (sorbitol/mannitol)	[1]	1.529	34.8	0.36	3
AAK86844	Atu1036	-	GTP-binding protein, Era family	[3]	1.510	8.1	-	3
AAK86355	Atu0542	Fla	flagellin	[4]	1.470	8.7	0.26	3
AAK91083	Atu6120	HupT	HNS-type DNA binding protein	[3]	1.465	26.8	-	2
AAK88274	Atu2549	AmeR	transcriptional regulator, TetR family	[7]	1.464	17.6	-	2
AAK87079	Atu1284	BirA	birA bifunctional protein	[3]	1.463	20.0	-	3
AAK87109	Atu1318	GatC	glutamyl-tRNA-Gln-amidotransferase chain C	[6]	1.452	35.4	-	2
AAK86042	Atu0224	CtpA	components of type IV pilus, pilin subunit	[4]	1.439	13.2	-	2
AAK86684	Atu0877	-	conserved hypothetical protein	[7]	1.436	17.1	-	2
AAK88480	Atu2765	-	transcriptional regulator, CarD family	[3]	1.417	7.8	-	3
AAK90805	Atu5430	-	hypothetical protein Atu5430	[7]	1.404	4.0	-	2
AAK89265	Atu4170	-	conserved hypothetical protein	[7]	1.404	2.7	-	3
AAK88510	Atu2797	ChiG	cobalamin biosynthesis protein	[2]	1.392	8.1	-	2
AAK88990	Atu4450	-	ABC transporter, nucleotide binding/ATPase protein (sugar)	[1]	1.384	15.0	-	3
AAL41485	Atu0466	-	hypothetical protein Atu0466	[7]	1.382	17.0	-	3
AAK89922	Atu3472	BkdA2	2-oxoisovalerate dehydrogenase beta subunit	[2]	1.375	18.5	0.31	3
AAK86438	Atu0631	MoaB	molybdenum cofactor biosynthesis protein B	[7]	1.368	4.0	-	3
AAK85925	Atu0106	CspA	cold shock protein	[3]	1.365	16.8	-	3
AAK87328	Atu1540	-	conserved hypothetical protein	[7]	1.362	8.3	-	2
AAK88459	Atu2744	-	C4-dicarboxylate binding protein	[7]	1.354	25.4	-	3
AAK85908	Atu0088	-	conserved hypothetical protein	[7]	1.352	20.7	-	3

down-regulated proteins (FC<0.75)

Accession	Annotation	Protein	Description	KEGG ontology (1)	$\Delta ybeY/WT$ ratio (2)	$\Delta ybeY/WT$ variability [%]	$\Delta hfq/WT$ ratio	replicates
AAK88379	Atu2658	GuaA	GMP synthase - glutamine amidotransferase protein	[5]	0.590	27.5	-	3
AAK89058	Atu4382	NirK	nitrite reductase, copper-containing	[2]	0.591	26.9	-	3
AAK90660	Atu5284	-	alpha-amylase	[6]	0.592	5.0	-	2
AAK90320	Atu3063	-	ABC transporter, nucleotide binding/ATPase protein	[1]	0.593	16.8	11.92	3
AAK88073	Atu2330	-	oxidoreductase	[7]	0.610	17.0	-	2
AAK90866	Atu5491	CatE	catalase C	[2]	0.638	14.5	-	3
AAK90867	Atu5492	-	conserved hypothetical protein	[7]	0.654	16.3	-	3
AAK88868	Atu4577	-	ABC transporter, substrate binding protein	[1]	0.662	7.0	12.94	3
AAK89929	Atu3465	-	metallo-beta-lactamase superfamily protein	[7]	0.662	0.9	-	2
AAK86796	Atu0987	-	DNA polymerase	[2]	0.662	16.8	-	2
AAK88128	Atu2391	-	ABC transporter, substrate binding protein (nitrate/sulfonate/taurine/bicarbonate)	[1]	0.667	4.3	9.82	3
AAK90472	Atu5096	-	conserved hypothetical protein	[7]	0.678	27.5	-	2
AAK90861	Atu5484	-	conserved hypothetical protein	[7]	0.686	8.0	-	3
AAK87480	Atu1707	-	conserved hypothetical protein	[3]	0.696	32.3	-	3
AAK90716	Atu5343	-	ABC transporter, substrate binding protein (oligopeptide)	[1]	0.703	5.6	6.40	3
AAK89856	Atu3542	Prp1	serine/threonine protein phosphatase I	[2]	0.705	22.2	-	2
AAK90661	Atu5285	GlgA	glycogen synthase	[2]	0.707	35.7	-	3
AAK86750	Atu0945	-	conserved hypothetical protein	[7]	0.710	39.0	-	2
AAK86800	Atu0991	-	cation efflux system component	[7]	0.711	4.1	-	2
AAK88755	Atu4695	-	ABC transporter, substrate binding protein (oligopeptide)	[1]	0.711	4.1	10.12	3
AAK90698	Atu5324	-	zinc-binding oxidoreductase	[7]	0.713	8.1	-	2
AAL44562	Atu3752	-	conserved hypothetical protein	[7]	0.719	10.8	-	3
AAK90868	Atu5493	-	hypothetical protein Atu5493	[7]	0.720	29.8	-	3
AAK87316	Atu1525	-	conserved hypothetical protein	[7]	0.721	17.6	-	3
AAK90608	Atu5237	-	ABC transporter, substrate binding protein (amino acid)	[1]	0.723	10.7	5.10	3
AAK86137	Atu0321	MutM	formamidopyrimidine-DNA glycosylase	[3]	0.723	21.5	-	3

AAK88731	Atu4719	DppA3	ABC transporter, substrate binding protein (dipeptide)	[1]	0.732	12.8	7.73	3
AAK87704	Atu1942	RpsS	30S ribosomal protein S19	[3]	0.733	2.5	-	3
AAK90512	Atu5139	BlcC	Zn-dependent gamma butyryl lactone lactonase	[7]	0.741	18.2	-	2
AAK88736	Atu4714	-	conserved hypothetical protein	[7]	0.742	0.5	-	2
AAK89127	Atu4312	SoxA	sarcosine oxidase alpha subunit	[2]	0.742	27.7	3.03	3
AAK89668	Atu3738	-	Na ⁺ /H ⁺ antiporter	[1]	0.745	16.4	-	2
AAK86736	Atu0931	-	penicillin binding protein	[2]	0.749	6.8	-	2

[1] Transporters [2] Enzymes [3] Transcription [4] Motility & Chemotaxis [5] Translation [6] Others [7] Unknown

Table S2. Bacterial strains and plasmids used in this study

Bacterial strains	Relevant characteristics	Reference
<i>Agrobacterium tumefaciens</i>		
C58	wild-type	C. Baron, Montreal, Canada
C58 + v	Km ^R ; wild-type with pBBsyn empty vector control	(3)
C58 Δhfq	deletion of <i>hfq</i> (<i>atu1450</i>) open reading frame (ORF)	(3)
C58 <i>hfq</i> ^{3xFLAG}	chromosomal integration of 3xFLAG tag sequence at <i>hfq</i> 3' end	(2)
C58 $\Delta ybeY$	deletion of <i>ybeY</i> (<i>atu0358</i>) open reading frame (ORF)	this study
C58 <i>ybeY</i> ^{3xFLAG}	chromosomal integration of 3xFLAG tag sequence at <i>hfq</i> 3' end	this study
C58 $\Delta hfq \Delta ybeY$	$\Delta hfq \Delta ybeY$ double deletion strain	this study
C58 $\Delta hfq \Delta ybeY$ + v	Km ^R ; $\Delta hfq \Delta ybeY$, with pBBsyn empty vector control	this study
C58 $\Delta hfq \Delta ybeY$ + <i>hfq</i>	Km ^R ; $\Delta hfq \Delta ybeY$, with pBBsyn_ <i>hfq</i> complementation plasmid	this study
C58 $\Delta hfq \Delta ybeY$ + <i>ybeY</i>	Km ^R ; $\Delta hfq \Delta ybeY$, with pBBsyn_ <i>ybeY</i> complementation plasmid	this study
C58 $\Delta hfq \Delta ybeY$ + <i>ybeY</i> ^{Ec}	Km ^R ; $\Delta hfq \Delta ybeY$, with pBBsyn_ <i>ybeY</i> ^{Ec} complementation plasmid	this study
C58 $\Delta hfq \Delta ybeY$ + <i>ybeY</i> Sm	Km ^R ; $\Delta hfq \Delta ybeY$, with pBBsyn_ <i>ybeY</i> Sm complementation plasmid	this study
C58 Δhfq + pBISN1	Km ^R ; <i>hfq</i> mutant with pBISN1	(3)
C58 $\Delta ybeY$ + pBISN1	Km ^R ; <i>ybeY</i> mutant with pBISN1	this study
<i>Escherichia coli</i>		
BL21 DE3	strain for pET-based protein expression	Novagen, Madison, USA
BL21 DE3, <i>ybeY</i> _C-His	Km ^R ; BL21 DE3 with pET24b_ <i>ybeY</i> _C-His	this study
BL21 DE3, <i>ybeY</i> _N-His	Km ^R ; BL21 DE3 with pET28b_ <i>ybeY</i> _N-His	this study
BL21 DE3, <i>hfq</i> _C-His	Km ^R ; BL21 DE3 with pET24b_ <i>hfq</i> _C-His	(3)
Plasmids		
pK19 <i>mobsacB</i>	Km ^R , suicide vector for mutagenesis	(4)
<i>ybeY</i> _up_3xFLAG_down	Km ^R , upstream region including <i>ybeY</i> ORF with additional 3' end 3xFLAG fusion and downstream region in pK19 <i>mobsacB</i>	this study
<i>ybeY</i> _up_dwn	Km ^R , up- and downstream region of <i>ybeY</i> for mutagenesis	this study
pBBsyn	Km ^R ; complementation vector	Novagen, Madison, USA

pBBSyn_ <i>hfq</i>	Km ^R ; pBBSyn derivate carrying the <i>hfq</i> gene for complementation	(3)
pBBSyn_ <i>ybeY</i>	Km ^R ; pBBSyn derivate carrying the <i>ybeY</i> (<i>atu0358</i>) gene for complementation	this study
pBBSyn_ <i>ybeY</i> ^{Ec}	Km ^R ; pBBSyn derivate carrying the <i>E. coli</i> K12 <i>ybeY</i> gene for complementation	this study
pBBSyn_ <i>ybeY</i> Sm	Km ^R ; pBBSyn derivate carrying the <i>S. meliloti ybeY</i> Sm (<i>smc01113</i>) gene for complementation	this study
pBISN1	Km ^R ; pBin19 derivate carrying T-DNA borders enclosing the <i>gusA</i> intron	(5)
pET24b_ <i>ybeY</i> _C-His	Km ^R ; pET24b derivate carrying the <i>ybeY</i> gene with a 6xHis tag a the 3' end	this study
pET28b_ <i>ybeY</i> _N-His	Km ^R ; pET28b derivate carrying the <i>ybeY</i> gene with a 6xHis tag a the 5' end	this study
pAC01_ <i>virB-lacZ</i>	Km ^R ; <i>virB1-lacZ</i> fusion (transcriptional) in pAC01	(6)
pET24b_ <i>hfq</i> _C-His	Km ^R ; pET24b derivate carrying the <i>hfq</i> gene with a 6xHis tag a the 3' end	(3)

Table S3. Oligonucleotides used in this study

Oligonucleotide	Sequence 5' to 3'
ybeY mutagenesis	
<i>ybeY</i> _up_PstI_fw	AAA <u>ACTGCAG</u> CCAACGCCCGTCATTCTGG
<i>ybeY</i> _up_SmaI_rv	AAA <u>CCCGGG</u> TGTCTTTACGTCAGTTGCCCTTCGG
<i>ybeY</i> _down_SmaI_fw	AAA <u>CCCGGG</u> AATTGTCTGGAGCCATGAACG
<i>ybeY</i> _down_EcoRI_rv	AAA <u>GAA</u> TTCGACCCGAACCTCCCGAAACG
ybeY 3xFLAG integration	
<i>ybeY</i> _up_SalI_rv	AAA <u>AGTCGAC</u> AAGCGGCTCCTGACCCGCG
<i>ybeY</i> _down_Acc65I_fw	AAA <u>AGGTACC</u> ATATTGTCTGGAGCCATGAACG
3xFLAG_SalI_fw	AAA <u>AGTCGAC</u> GACTACAAAGACCATGACGGTG
3xFLAG_Acc65I_rv	AAA <u>AGGTACC</u> TCTATTTATCGTCGTCATCTTTGTAG
YbeY His-tag fusion proteins	
<i>ybeY</i> _C_fw_EcoRI	<u>GAATTC</u> ATGGCGGCTCTGGATATTCAAATCAGC
<i>ybeY</i> _C_rv_SalI	<u>GTCGACA</u> AGCGGCTCCTGACCCGCGT
<i>ybeY</i> _N_fw_NdeI	<u>CATATG</u> ATGGCGGCTCTGGATATTCAAATCAGC
<i>ybeY</i> _N_rv_XhoI	<u>CTCGAGT</u> TAAAGCGGCTCCTGACCCGCG
Complementation with ybeY homologs from <i>S. meliloti</i> and <i>E. coli</i>	
<i>smc01113</i> _fw_XbaI	<u>TCTAGA</u> AATGACGGCATTGGACATTCAAGATCAGC
<i>smc01113</i> _rv_SacI	<u>GAGCTC</u> TTAATGCGGGGGTTGGTCCCCG
<i>Ec_ybeY</i> _fw_XbaI	<u>TCTAGA</u> AATGAGTCAGGTGATCCTCGATTTACAACCTG
<i>Ec_ybeY</i> _rv_SacI	<u>GAGCTC</u> TATTCTTTCTCGGCAATGTACGGATCC
5' RACE	
adapter oligo	GTCAGCAATCCCTAACGAG (<i>GAG ribonucleotides</i>)
5'RACE_rv_1	GGTAGGCCTTTACCCACC
5'RACE_rv_2	GGGCGTATGCGGTATTAATTCC

Restriction sites are underlined. T7 promoter sequences are written in bold letters.

References

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