Role of *Escherichia coli* YbeY, a highly conserved protein, in rRNA processing.

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SUPPLEMENTARY INFORMATION

3 Supplementary Figures.

2 Supplementary Tables.

Supplementary References.

Figure S1. Sensitivity of the Δ*ybeY* mutant to stresses (**A**) deoxycholate and (**B**) hydrogen peroxide (H_2O_2). The curve for the Δ*ybeY* mutant with empty vector only (Δ*ybeY*+vector) is shown on each plot for clarity. UPF0054 homologs: *ybeY* (*E. coli*), *yqfG* (*B. subtilis*) and *SMc01113 (S. meliloti*). MC4100+vector (■), Δ*ybeY*+vector (●), Δ*ybeY*+p*ybeY* (▲), Δ*ybeY*+p*yqfG* (▼) and Δ*ybeY*+p*SMc01113* (◄). "p" indicates that the gene indicated is expressed from a plasmid. "+vector" indicates the strain carried the vector only as a control.

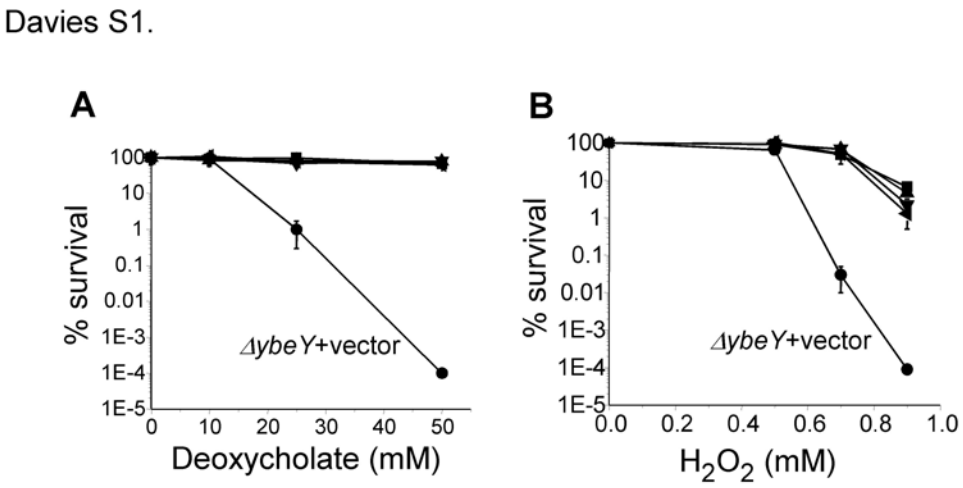

Figure S2. Complementation of the *S. meliloti SMc01113*::mTn*5* mutant by *ybeY*. (**A**) *S. meliloti* strains were diluted to an OD_{600} of 0.01 in LB and grown at 30 ^{o}C. Growth over time was monitored by OD_{600}. (**B-C**) Sensitivity of *S. meliloti* strains to stresses. Cells were serially diluted and plated on LB plates containing increasing concentrations of (**B**) deoxycholate and (**C**) cefotaxim. Colonies were counted after 96 h of growth at 30 ^{o}C. Rm1021+vector (■), *SMc01113*::mTn*5*+vector (●) and *SMc01113*::mTn*5*+p*ybeY* (▲). "p" indicates that the gene indicated is expressed from a plasmid. "+vector" indicates the strain carried the vector only as a control.

Davies S2.

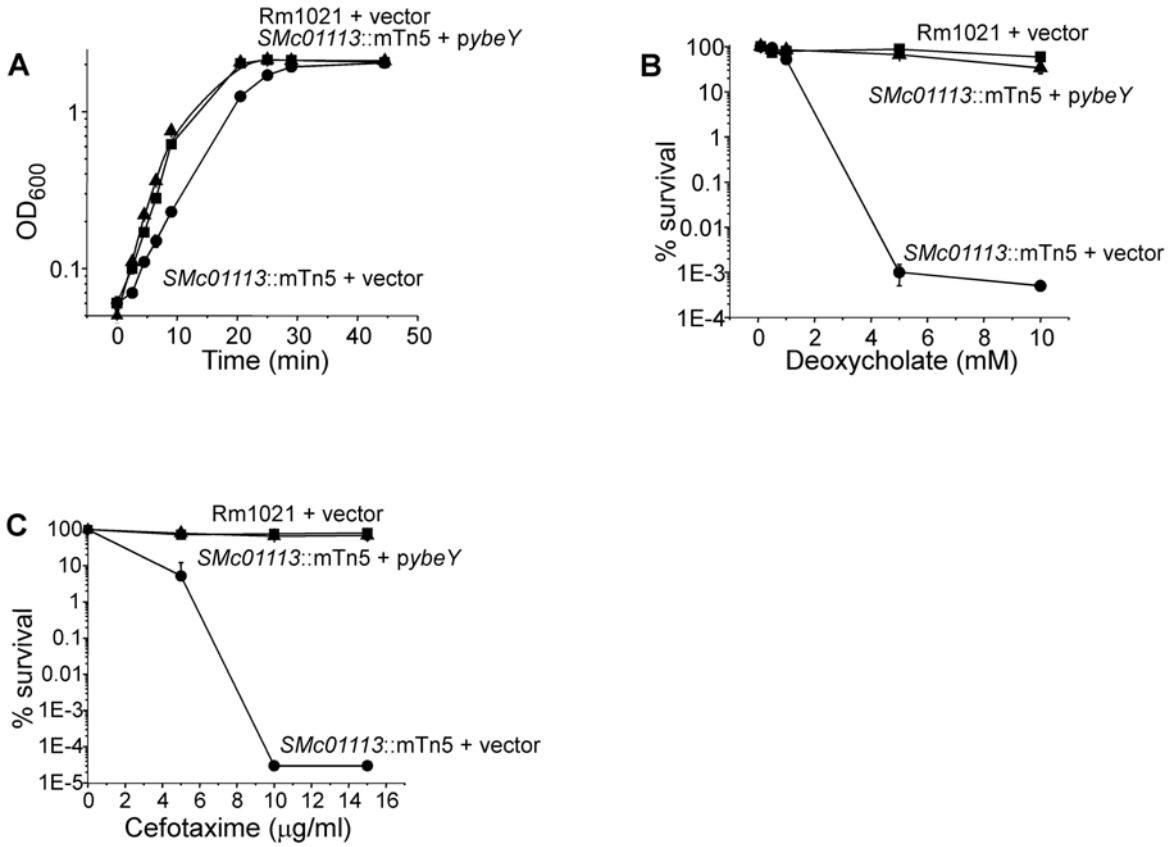

Figure S3. Polysome profiles for (**A**) MC4100+vector, (**B**) Δ*ybeY*+vector and (**C**) Δ*ybeY*+p*ybeY*. Cell extracts were separated on a 10 – 40% sucrose gradient. The gradient was fractionated and the A_{260} of each sample was determined. The positions of polysomes, 70S, 50S and 30S ribosomes are indicated. "p" indicates that the gene indicated is expressed from a plasmid. "+vector" indicates the strain carried the vector only as a control. (**D-F**) Agarose gel electrophoresis of total rRNA extracted from each indicated strain. The parental strain MC4100 is shown in each case as a control. The positions of 23S, 17S, 16S and 16S* rRNAs are indicated. (**D**) Complementation of the Δ*ybeY* mutant strain by *ybeY*. "p" indicates that the gene indicated is expressed from a plasmid. (**E**) Sensitivity of the Δ*ybeY* mutant to temperature. (**F**) Analysis of rRNA from the Δ*ybeY* and the rne^{ts} mutants and the Δ*ybeY* rne^{ts} double mutant. (**G**) Western blot showing relative protein expression levels of *ybeY* alleles. The *ybeY* complementation plasmid carries a C-terminal FLAG tag (lanes 3-5). In a separate strain, we have integrated a FLAG tag at the C-terminus of the genomic locus of *ybeY* (lane 2) to monitor endogenous levels of YbeY. Addition of a C-terminal FLAG tag has no observable effect on YbeY function (data not shown). Immunoblotting with an anti-FLAG antibody (two exposures are shown) shows that WT and YbeY H114A are expressed at equivalent levels (lanes 3 and 4) from the complementation plasmid. The R59A YbeY is expressed at lower levels than WT YbeY (lanes 3 and 5) from the complementation plasmid. However, expression of plasmid encoded R59A YbeY (lane 5) is still much higher than from the endogenous *ybeY* locus (lane 2). Thus all plasmid encoded *ybeY* alleles are expressed at higher levels than from the endogenous locus. RpoB is used as a loading control.

Davies S3.

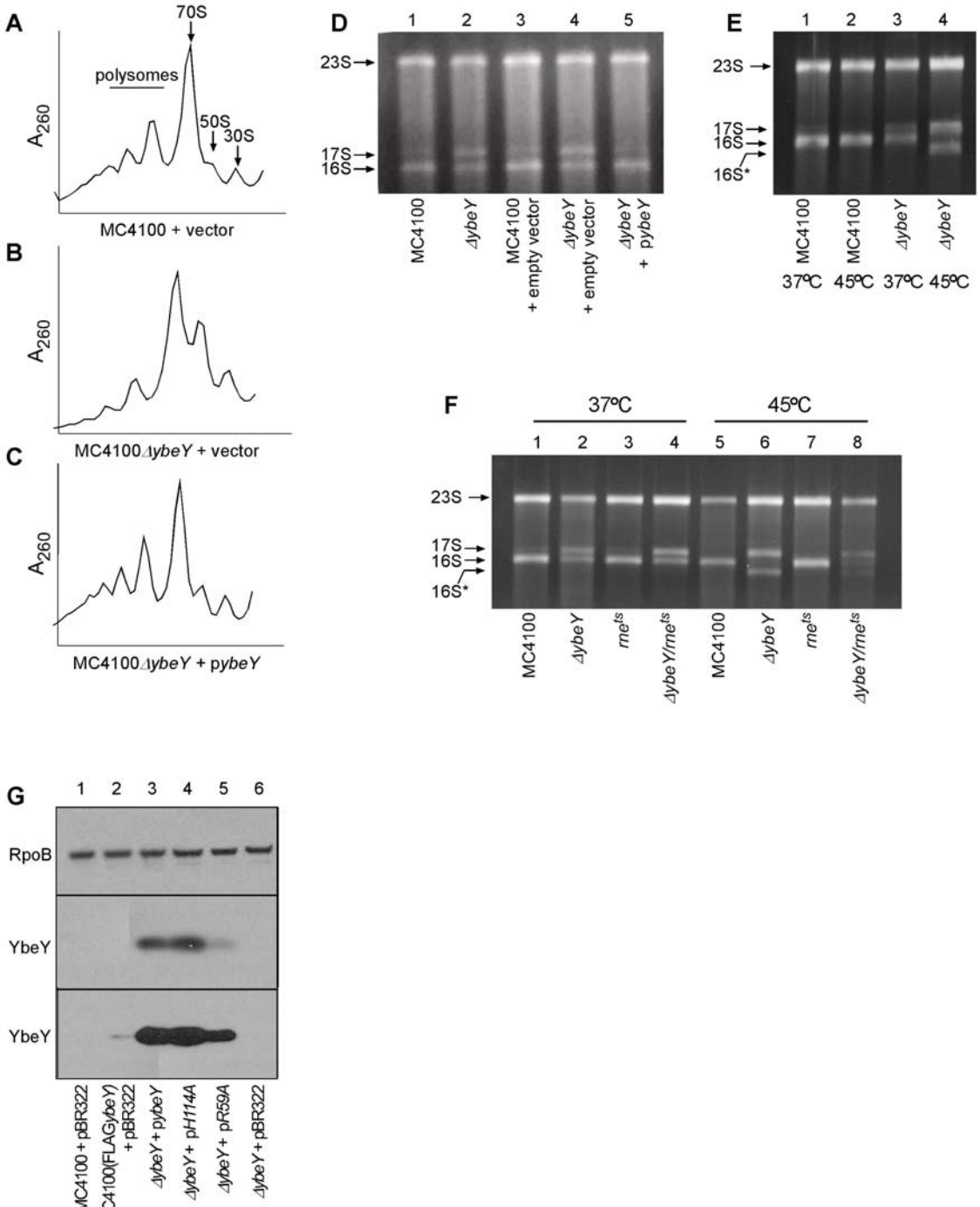
A
70S
polysomes
50S
30S
A260
MC4100 + vector
B
A260
MC4100ΔybeY + vector
C
A260
MC4100ΔybeY + pybeY
D
1 2 3 4 5
23S
17S
16S
MC4100
ΔybeY
MC4100 + empty vector
ΔybeY + empty vector
ΔybeY + pybeY
E
1 2 3 4
23S
17S
16S
16S*
MC4100
MC4100
ΔybeY
ΔybeY
37ºC 45ºC 37ºC 45ºC
F
37ºC
45ºC
1 2 3 4 5 6 7 8
23S
17S
16S
16S*
MC4100
ΔybeY
rne^ts
ΔybeY/rne^ts
MC4100
ΔybeY
rne^ts
ΔybeY/rne^ts
G
1 2 3 4 5 6
RpoB
YbeY
YbeY
MC4100 + pBR322
MC4100(FLAGybeY) + pBR322
ΔybeY + pybeY
ΔybeY + pH114A
ΔybeY + pR59A
ΔybeY + pBR322

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Table S1. Bacterial strains and plasmids used in this study.

Strain/plasmid	Relevant genotype and property	Source
Strain		
MC4100	F⁻ *araD139* Δ*lacU169* Δ*relA1 rpsL150 thi mot flb5301 deoC7 ptsF25 rbsR*	Laboratory stock
MG1655	F- λ- *ilvG- rfb*-50 *rph*-1	Laboratory stock
Δ*ybeY*::cat^R	*ybeY* replaced with cat^R cassette in MC4100	This study
Δ*ybeY*	*ybeY* clean deletion in MC4100	This study
BWD10	MC4100 carrying pBR322	This study
BWD11	Δ*ybeY* carrying pBR322	This study
BWD12	Δ*ybeY* carrying pBWD1	This study
BWD13	Δ*ybeY* carrying pBWD2	This study
BWD14	Δ*ybeY* carrying pBWD3	This study
BWD15	Δ*ybeY* carrying pBWD4	This study
Rm1021	SU47 Sm^R	(Ausubel, 1991)
BWD16	Rm1021 carrying pMSO3	This study
GWBD12	Rm1021 *SMc01113*::mTn*5* transduced	(Davies and Walker, 2008)
BWD17	GWBD12 carrying pMSO3	This study
BWD18	GWBD12 carrying pBWD5	This study
CA244I⁻II⁻	CA244, Δ*rnb201*::tet^R	(Cheng and Deutscher, 2005)
CA244D⁻	CA244, *rnd*⁻	(Reuven and Deutscher, 1993)
CA244T⁻	CA244, *rnt*⁻, kan^R	(Reuven and Deutscher, 1993)

CA244PH$^-$	CA244, *rph*$^-$, kan^R	(Kelly *et al.*, 1992)
CA265R$^-$	CA265, Δ*rnr*::cat^R	(Cheng *et al.*, 1998)
SK5695	*rne-1*, Tc^R	(Babitzke and Kushner, 1991)
BWD19	MG1655 Δ*pnp*::kan^R	(Baba *et al.*, 2006)
BWD20	MC4100 Δ*rnd*::kan^R	This study
BWD21	MC4100 *rph*$^-$, kan^R	This study
BWD22	MC4100 *rnt*$^-$, kan^R	This study
BWD23	MC4100 Δ*rnc*::cat^R	This study
BWD24	MC4100 Δ*cafA*::kan^R	This study
BWD25	MC4100 Δ*pnp*::kan^R	This study
BWD26	MC4100 Δ*rnr*::cat^R	This study
BWD27	Δ*ybeY* Δ*rnd*::kan^R	This study
BWD28	Δ*ybeY* *rph*$^-$ kan^R	This study
BWD29	Δ*ybeY* Δ*rnc*::cat^R	This study
BWD30	Δ*ybeY* Δ*rnr*::cat^R	This study
BWD31	Δ*ybeY* Δ*caf*::kan^R	This study
BWD32	Δ*ybeY* Δ*pnp*::kan^R	This study
BWD33	Δ*ybeY*::cat^R *rne-1*, tet^R	This study
BWD34	MC4100 carrying pSG25	This study
BWD35	MC4100 carrying pSG163	This study
BWD36	MC4100 carrying pSG853	This study
BWD37	MC4100 carrying pSG3/4	This study
BWD38	MC4100 carrying plac7	This study

BWD39	MC4100 carrying plac10	This study
BWD40	Δ*ybeY* carrying pSG25	This study
BWD41	Δ*ybeY* carrying pSG163	This study
BWD42	Δ*ybeY* carrying pSG853	This study
BWD43	Δ*ybeY* carrying pSG3/4	This study
BWD44	Δ*ybeY* carrying plac7	This study
BWD45	Δ*ybeY* carrying plac10	This study
BWD46	Δ*ybeY* carrying pBWD5	This study
BWD47	Δ*ybeY* carrying pBWD6	This study
BWD48	Δ*ybeY* carrying pBWD7	This study
BWD49	Δ*ybeY* carrying pBWD8	This study
BWD50	Δ*ybeY* carrying pBWD9	This study
BWD51	Δ*ybeY* carrying pBWD10	This study
BWD52	Δ*ybeY* carrying pBWD11	This study
BWD53	Δ*ybeY* carrying pBWD12	This study
BWD54	Δ*ybeY* carrying pBWD13	This study
BWD55	MC4100 with YbeY carrying a C-terminal FLAG tag	This study
Plasmid		
pBR322	amp^R, tet^R	(Bolivar *et al.*, 1977)
pBWD1	pBR322 expressing *ybeY*	This study
pBWD2	pBR322 expressing *SMc01113*	This study
pBWD3	pBR322 expressing *yqfG*	This study

pMS03	Spc^R	(Barnett *et al.*, 2000)
pBWD4	pMSO3 carrying *ybeY*	This study
pSG25	WT *lacZ*	(O'Connor *et al.*, 1992)
pSG163	*lacZ* carrying UAG interruption	(O'Connor *et al.*, 1992)
pSG853	*lacZ* carrying UAA interruption	(O'Connor *et al.*, 1992)
pSG3/4	*lacZ* carrying UGA interruption	(O'Connor *et al.*, 1992)
plac7	*lacZ* carrying +1 frameshift	(O'Connor *et al.*, 1992)
plac10	*lacZ* carrying -1 frameshift	(O'Connor *et al.*, 1992)
pBWD5	pBWD1 N55A	This study
pBWD6	pBWD1 R59A	This study
pBWD7	pBWD1 D62A	This study
pBWD8	pBWD1 T65A	This study
pBWD9	pBWD1 S69A	This study
pBWD10	pBWD1 H114A	This study
pBWD11	pBWD1 H118A	This study
pBWD12	pBWD1 D123A	This study
pBWD13	pBWD1 H124A	This study

Table S2. Primers and probes used in this study.

λ-red primers (5´-3´)
YbeY for: gctggcagcagaacgcaagcgcgaagaacaggaacaaaaatgagtcaggtgtgtaggctggagctgcttc
YbeY rev: gttaatcaccaacggcggggacgtctgccagtcaaatgcctggcaaattaatatgaatatcctccttagt

YbeY FLAG for: agattatgcttgctctgggctatgaggatccgtacattgccgagaaagaagactacaaggacgac gatgacaaataataagtgtaggctggagctgcttc

YbeY FLAG rev: gtcgttaatcaccaacggcggggacgtctgccagtcaaatgcctggcaaaatatgaatatcctccttagt

Complementation primers (5´-3´)

YbeY for: atagctagcgaagaacaggaacaaaaatgagtcagg

YbeY rev: atagtcgacttattattctttctcggcaatgtacggatc

YbeY FLAG rev: atagtcgacttattatttgtcatcgtcgtccttgtagtcttctttctcggcaatgtacggatc

YbeY pMSO3 for: atactcgaggaggatacaaaaatgagtcaggtgatcctc

YbeY pMSO3 rev: ataggtaccttattattctttctcggcaatgtacggatc

YqfG for: atagctagcgaggctactaagaaggtgaaatagatgagt

YqfG rev: atagtcgaccactcatttctatgatcttttgagtcc

SMc01113 for: atagctagcaggaggaaacgatgacggcattggacattcagatcagc

SMc01113 rev: atagtcgacttaatgcgggggttgg

Northern probes (5´-3´)

17S 5`: gtgggcactcgaagatacggattcttaacgtcg

17S 3´: tgtgtgagcacttcaaagttcgcttctttaagg

Northern probes for hybridization after RNase H cleavage at the 3´-terminus (5´-3´)

CK_5S_3´mature: atgcctggcagttccctactctcgc

CK_23S_3´mature: aaggttaagcctcacggttcattag

CK_16S_3´mature: taaggaggtgatccaaccgcaggttcccct

Primer extension primers (5´-3´)

16S 5´: cgacttgcatgtgttagg

23S 5´: gggcatccaccgtgtacgcttagtcg

5S 5´: ggggtcaggtgggaccaccgcgcta

Site directed mutagenesis primers (5´-3´)

N55A for: agccacagtctggctctgacctatcgcggtaaggat

N55A rev: atccttaccgcgataggtcagagccagactgtggct

R59A for: aatctgacctatgccggtaaggataagccgaccaacgtg

R59A rev: cacgttggtcggcttatccttaccggcataggtcagatt

D62A for: acctatcgcggtaaggctaagccgaccaacgtgctc

D62A rev: gagcacgttggtcggcttagccttaccgcgataggt

T65A for: aaggataagccggccaacgtgctctccttcccg

T65A rev: cgggaaggagagcacgttggccggcttatcctt

S69A for: accaacgtgctcgccttcccgtttgaagtgccgcct

S69A rev: aggcggcacttcaaacgggaaggcgagcacgttggt

H114A for: catatggtggtggccggcagtctgcatttgttaggt

H114A rev: acctaacaaatgcagactgccggccaccaccatatg

H118A for: gtgcacggcagtctggctttgttaggttacgatcacatc

H118A rev: gatgtgatcgtaacctaacaaagccagactgccgtgcac

D123A for: catttgttaggttacgctcacatcgaagatgacgaagca

D123A rev: tgcttcgtcatcttcgatgtgagcgtaacctaacaaatg

H124A for: ttgttaggttacgatgccatcgaagatgacgaagca

H124A rev: tgcttcgtcatcttcgatggcatcgtaacctaacaa

Supplementary References

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