Molecules and Cells

Supplementary Table S1. Oligonucleotides used in this study

Name	Sequence (5' to 3')
For Northern blotting	
DsrA_NP	GTTACACCAGGAAATCTGATGTGTT
lacZsdR1939	TATTCGCTGGTCACTTCGATGG
rpoS_NP	CTTCATTTA AATCATGAACTTTCAGCGTATTCTGACTCAT
For in vitro transcription	
DsrA_F_T7	TAATACGACTCACTATAGGAACACATCAGATTTCCTGGTG
DsrA_R	AAATCCCGACCCTGAGGGGG
lacZsdF1713_T7	GTGTAATACGACTCACTATAGGGGTCTGGGACTGGGTGGATCAG
lacZsdR1978	AAATCCCGACCCTGAGGGGG
For qRT-PCR	
rpoS:5'ORF_FW	GAAGATGCGGAATTTGATGAGAAC
rpoS:5'ORF_RV	AGTTCCTCTTCGGCCAAATC
rpoS:ORF_FW	ACCCGTACTATTCGTTTGCC
rpoS:ORF_RV	ATCTCTTCCGCACTTGGTTC
lacZsdF1713	GTCTGGGACTGGGTGGATCAG
lacZsdR1939	TATTCGCTGGTCACTTCGATGG
rrsA_968F	AACGCGAAGAACCTTAC
rrsA_1387R	CGGTGTGTACAAGGCCCGGG

Supplementary Table S2. Half-lives of the *rpoS* mRNA

strain	Half-lives (min)[a]	
	Vector	pDsrA
hfq^{+}	1.26 ± 0.42	2.08 ± 0.58
hfq^{-}	0.83 ± 0.16	1.69 ± 0.52

[a]Half-lives were determined by linear regression analysis from the data presented in Supplementary Figure S3. We assumed that the disappearance of *rpoS* mRNA after rifampicin treat followed a first-order decay. Values are means ± SD for three independent experiments.

Supplementary Figure S1

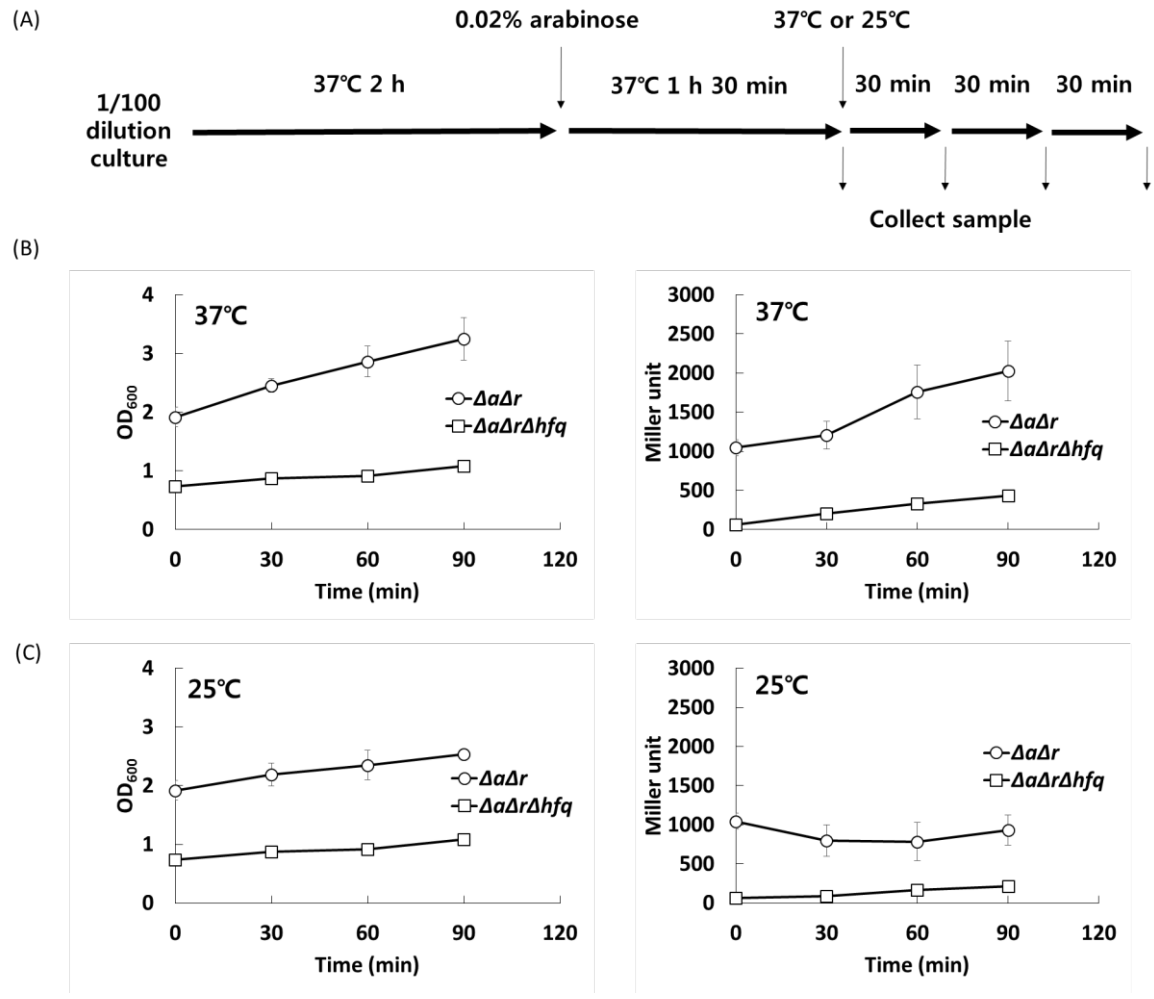

Figure S1. Up-regulation of *rpoS* translation by endogenous DsrA in *hfq*$^{+}$ and *hfq*$^{-}$ cells. (A) Schematic diagram of experimental conditions. Three colonies for each strain were cultured in LB medium containing ampicillin (100 μg mL^{-1}) and the overnight culture was diluted to 1:100 and cultured with the fresh medium. 0.02% arabinose at 37°C and IPTG were added at 2 h and 3.5 h, respectively, and the culture was incubated further for 30 min and 60 min at 37°C or 25°C. Cells grown at 37°C (B) and 25°C (C) were assayed for LacZ activity (Miller unit). The OD_{600} of cell cultures was also measured. The indicated values were calculated from at least three independent experiments (error bars represent standard deviation). *ΔaΔr, arcZ*$^{-}$ *dsrA*$^{+}$ *rprA*$^{-}$ *hfq*$^{+}$; *ΔaΔrΔhfq, arcZ*$^{-}$ d*srA*$^{+}$ *rprA*$^{-}$ *hfq*$^{-}$.

Supplementary Figure S2

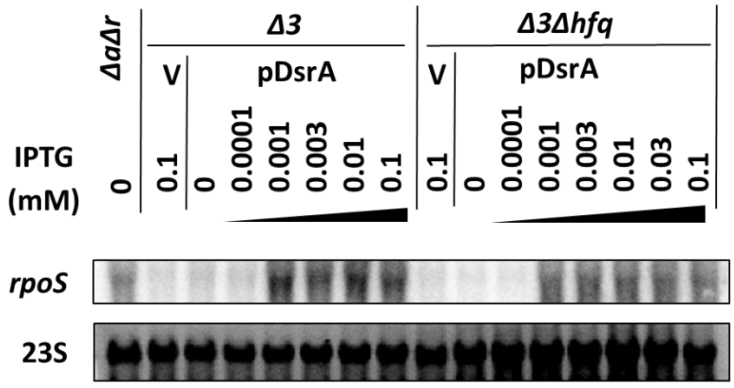

Figure S2. Northern analysis of effects of DsrA on *rpoS* mRNA accumulation in *Δ3* and *Δ3Δhfq* cells. Total cellular RNA was prepared from IPTG-treated cells grown at 37°C, and subjected to Northern blot analysis. Cells containing pDsrA were treated with IPTG at increasing concentrations from 0 to 0.1 mM. The *rpoS* mRNA was probed with an anti-*rpoS* ORF oligonucleotide and the 23S rRNA was detected as a loading control. *Δ3, arcZ*$^-$ *dsrA*$^-$ *rprA*$^-$ *hfq*$^+$; *Δ3Δhfq, arcZ*$^-$ *dsrA*$^-$ *rprA*$^-$ *hfq*$^-$; *ΔaΔr, arcZ*$^-$ *dsrA*$^+$ *rprA*$^-$ *hfq*$^+$; V, vector control.

Supplementary Figure S3

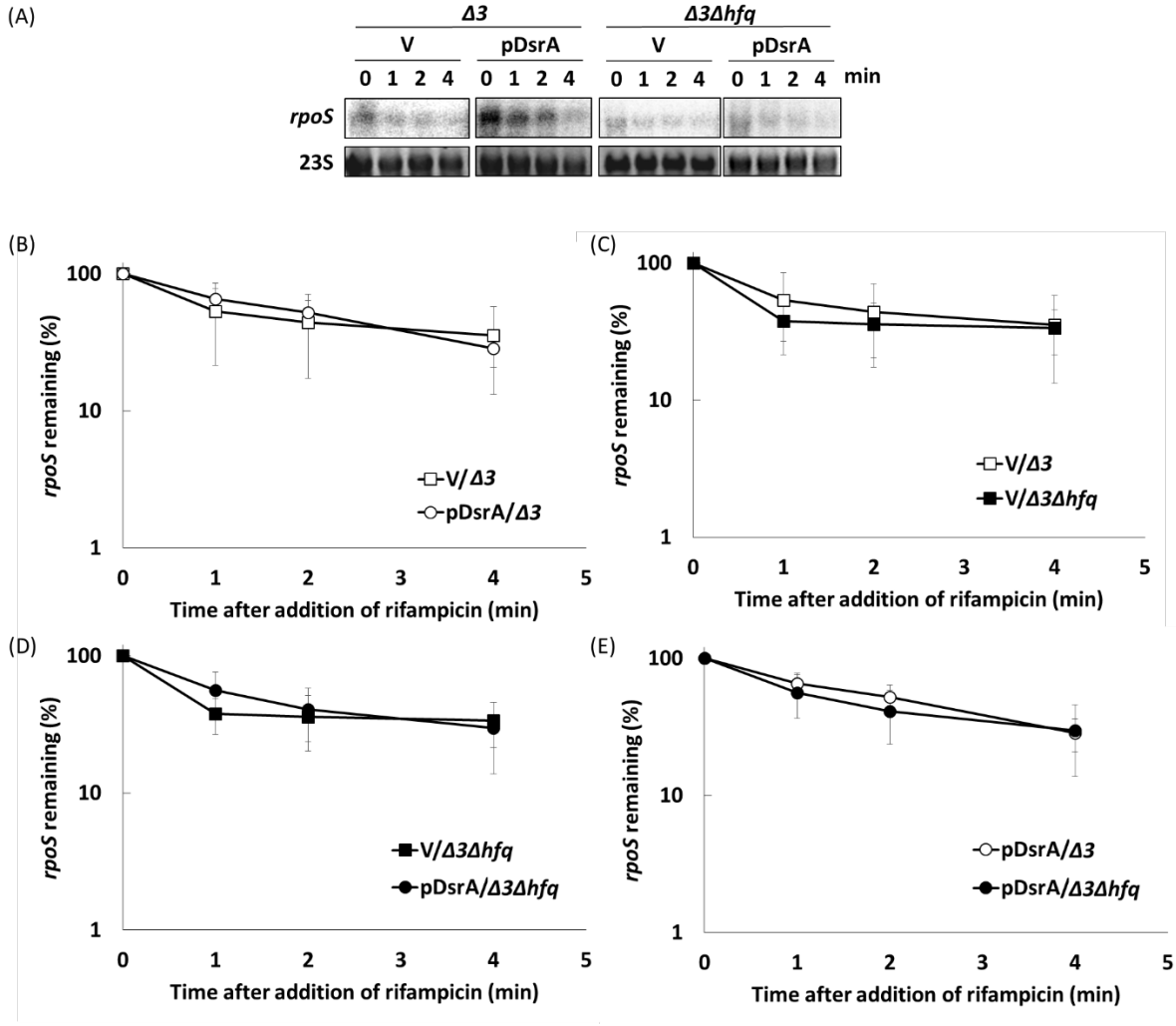

Fig. S3. Effects of DsrA on the stability of the *rpoS* mRNA in *hfq*$^{+}$ and *hfq*$^{-}$ cells. Total cellular RNA was prepared from 0.1 mM IPTG-induced DsrA-expressing cells grown at 37°C, at the indicated times after rifampicin treatment. (A) Cellular levels of *rpoS* mRNA were measured using Northern blot analysis. *rpoS* mRNA was probed with an anti-*rpoS* oligonucleotide and the 23S rRNA was detected as a loading control. (B to E) The % *rpoS* mRNA remaining was plotted on a semi-log scale as a function of time. Values are means ± SD; n = 3. PM1409*Δ3* cells containing control vector and pDsrA (B), PM1409*Δ3* and PM1409*Δ3Δhfq* cells containing control vector (C), PM1409*Δ3Δhfq* cells containing control vector and pDsrA (D), and PM1409*Δ3* and PM1409*Δ3Δhfq* cells containing pDsrA (E). *Δ3, arcZ*$^{-}$ *dsrA*$^{-}$ *rprA*$^{-}$ *hfq*$^{+}$; *Δ3Δhfq, arcZ*$^{-}$ *dsrA*$^{-}$ *rprA*$^{-}$ *hfq*$^{-}$; V, vector control. At least three independent measurements were performed for each strain (error bars represent standard deviation).

Supplementary Figure S4

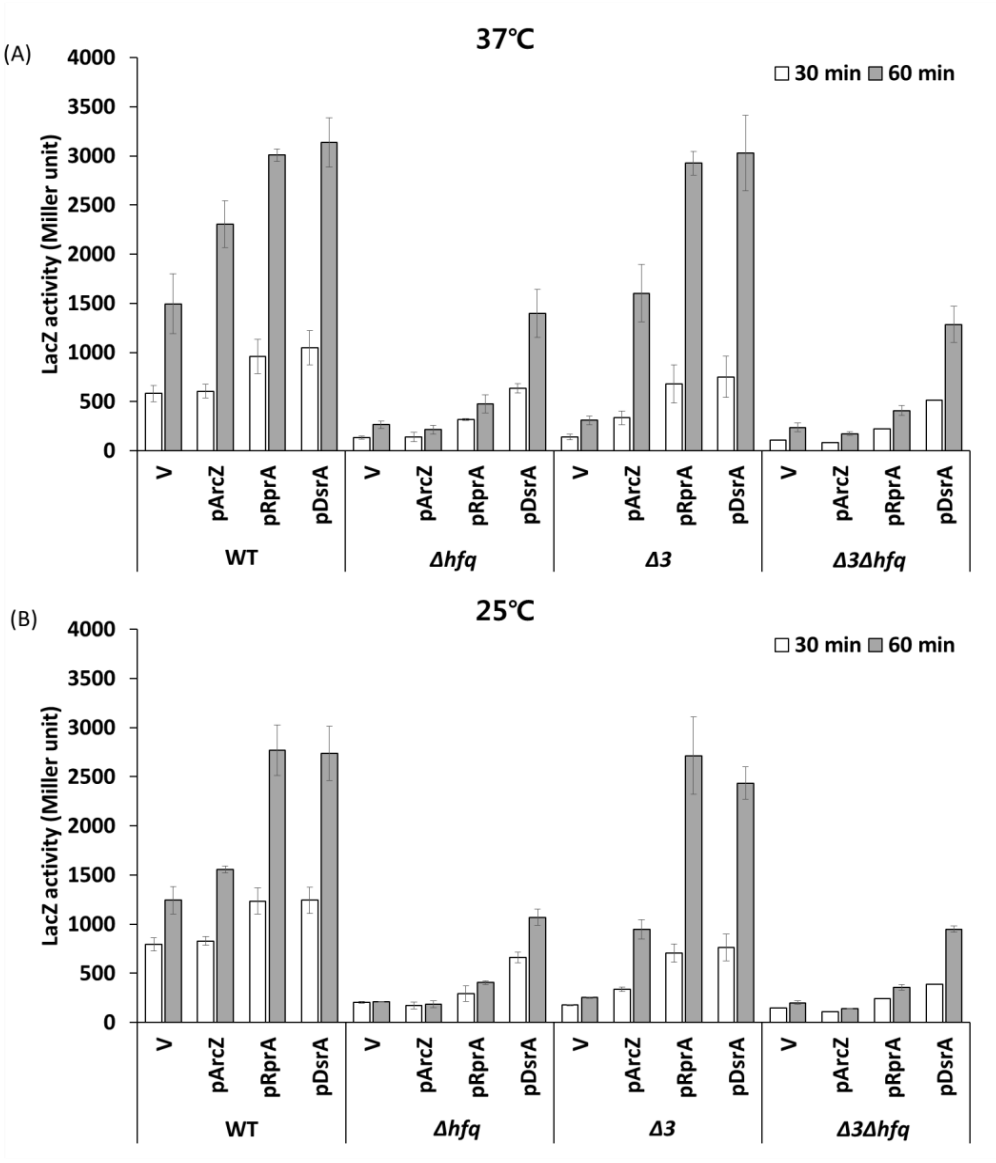

Fig. S4. Stimulation of *rpoS* translation by DsrA overexpression in the absence of Hfq at 37°C and 25°C. Cells were cultured with following condition and LacZ activity was measured. Three colonies for each strain were cultured in LB medium containing ampicillin (100 μg mL^{-1}) and the overnight culture was diluted to 1:100 and cultured with the fresh medium. 0.02% arabinose at 37°C and IPTG were added at 2 h and 3.5 h, respectively, and the culture was incubated further for 30 min and 60 min at 37°C or 25°C. *rpoS* activation by overexpression of sRNAs in WT, *Δhfq, Δ3* and *Δ3Δhfq* (lacking all three *rpoS*-activating sRNAs) was assayed by LacZ expression. Cells grown at 37°C (A), and 25°C (B). Control vector, pHMB1 (V). Plasmids pArcZ, pRprA, and pDsrA overexpress DsrA, RprA, and ArcZ, respectively. WT*, arcZ*$^{+}$ *dsrA*$^{+}$ *rprA*$^{+}$ *hfq*$^{+}$; *Δhfq, arcZ*$^{+}$ *dsrA*$^{+}$ *rprA*$^{+}$ *hfq*$^{-}$; *Δ3, arcZ*$^{-}$ *dsrA*$^{-}$ *rprA*$^{-}$ *hfq*$^{+}$; *Δ3Δhfq, arcZ*$^{-}$ *dsrA*$^{-}$ *rprA*$^{-}$ *hfq*$^{-}$.

Supplementary Figure S5

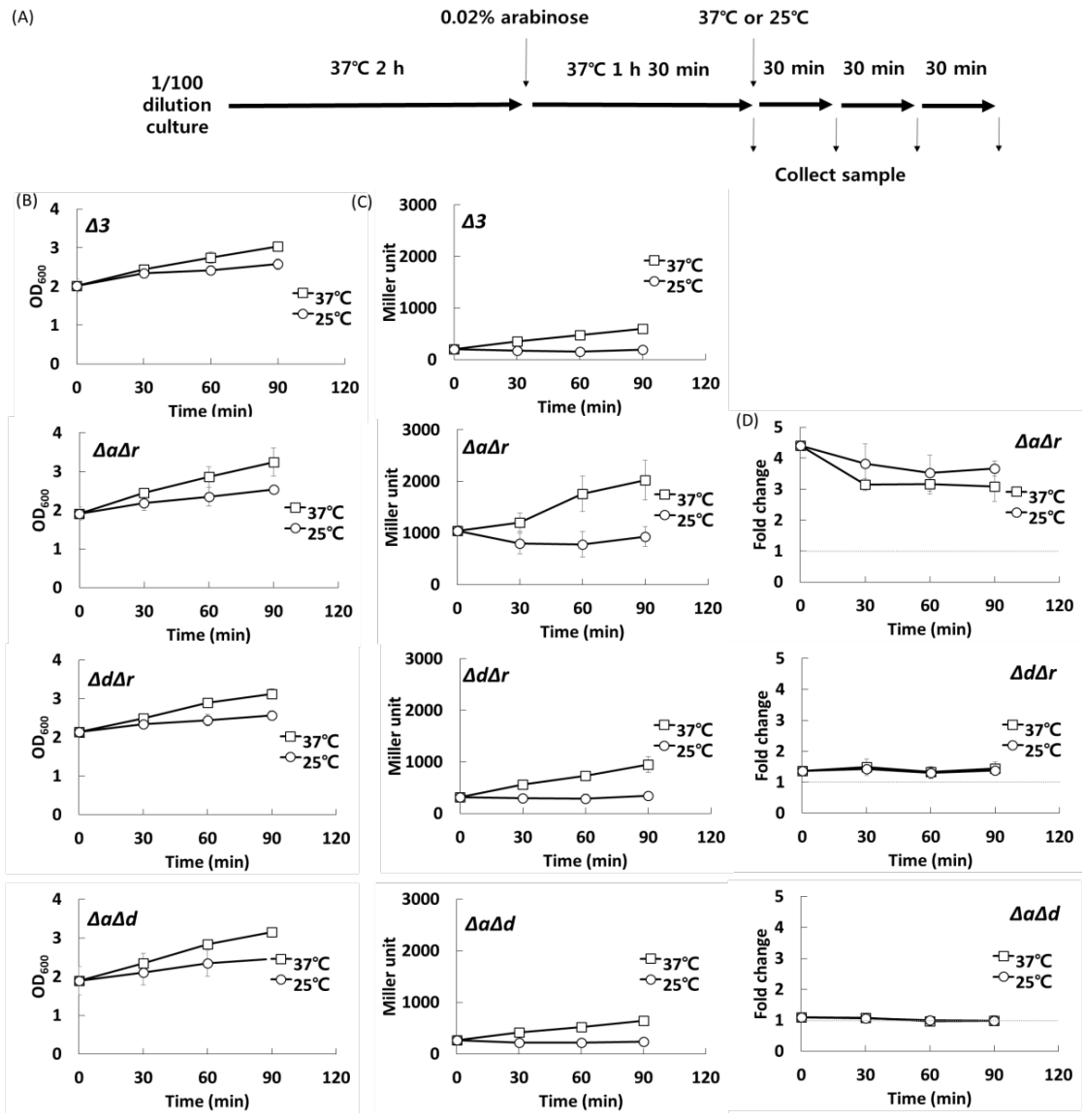

Figure S5. Up-regulation of *rpoS* translation by endogenous *rpoS*-activating sRNAs at 37°C and 25°C. (A) Schematic diagram of experimental conditions. Three colonies for each strain were cultured in LB medium containing ampicillin (100 μg mL^{-1}) and the overnight culture was diluted to 1:100 and cultured with the fresh medium. 0.02% arabinose at 37°C and IPTG were added at 2 h and 3.5 h, respectively, and the culture was incubated further for 30 min and 60 min at 37°C or 25°C. Cells grown at 37°C (B) and 25°C (C) were assayed for LacZ activity (Miller unit). The OD_{600} of cell cultures was also measured. OD_{600} values (B), LacZ activity (C), and fold changes in LacZ activities of cells expressing only one *rpoS*-activating sRNA relative to *Δ3* cells (D). The indicated values were calculated from at least three independent experiments (error bars represent standard deviation). *Δ3, arcZ⁻ dsrA⁻ rprA⁻ hfq⁺*; *ΔaΔr, arcZ⁻ dsrA⁺ rprA⁻ hfq⁺*; *ΔaΔd, arcZ⁻* d*srA⁻ rprA⁺ hfq⁺*; *ΔdΔr, arcZ⁺ dsrA⁻ rprA⁻ hfq⁺*.