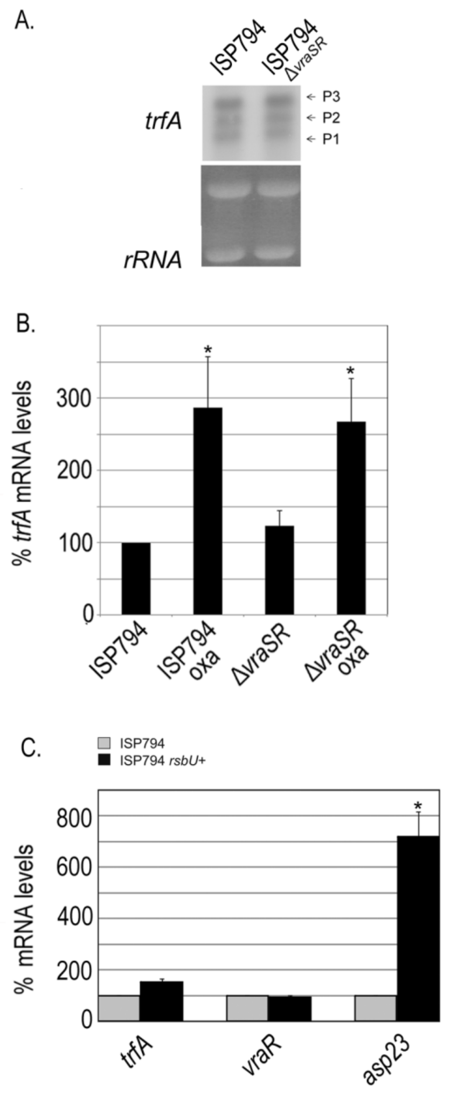

FIG. S1. Effect of *VraR* and σ^{B} transcriptional regulators on *trfA* transcription. A) Northern blot analysis of *trfA* transcripts in ISP794 and ISP794Δ*vraSR* strains, using a radiolabeled ^{32}P RNA *trfA*-specific probe. Arrows indicate the three different *trfA* transcripts as described in Fig 4. **B)** Effect of *vraSR* mutation on oxacillin-dependent *trfA* induction. Steady-state levels of *trfA* transcripts were determined by qRT-PCR and normalized to 16S rRNA, in strains ISP794 and ISP794Δ*vraSR* in the presence or absence of 1 µg/ml oxacillin (oxa). Values represent the mean ± SEM of at least three independent experiments. (*) Represents results significantly different (Student's two tailed *t*-test, *p* <0.05) compared with ISP794. **C)** Steady-state levels of *trfA*, *vraR* and *asp23* transcripts were determined by qRT-PCR and normalized to 16S rRNA, in strains ISP794 and ISP794 (*rsbU*$^{+}$). Values represent the mean ± SEM of at least three independent experiments.

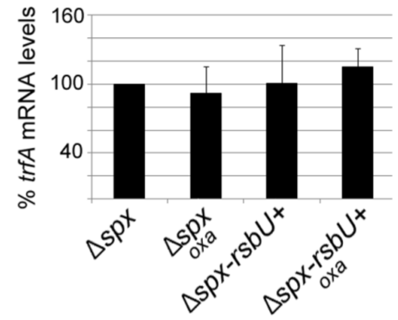

FIG. S2. Effect of *rsbU*$^{+}$ restoration on *spx*-dependent *trfA* transcription. Steady-state levels of *trfA* transcripts were determined by qRT-PCR and normalized to 16S rRNA. The mRNA levels of mutant Δ*spx* were compared to Δ*spx-rsbU*$^{+}$ in the presence or absence of oxacillin addition. Oxacillin induction of *trfA* transcription in wild-type strain was used as positive control for cell-wall stress (data not shown). Values represent the mean ± SEM of three independent experiments.

TABLE S1. Oligonucleotide PCR primers used in this study.

Gene/Mutant/Protein	**Primer**	**Primer sequence (5'-3')**[1]
TrfA Northern probe	*Kpn1-trfA*	CGGGTACCAAGGAGGAACAATCATGAGAATAGAACGAGTAGATGAT
	Pst1-trfA	AAAACTGCAGTATCGTTATTCAGTTGTCTCTGG
5'RACE	*trfA*- Pst1	AAAACTGCAGTTATTCAGTTGTCTCTGGAAAA
	trfA- nested	GATTTAGAAATTGTGACTTCGACACCTTTTTC
TrfA-intein recombinant protein	*Kpn-Nde1 trfA* E09	GGGGTACCCATATGAGAATAGAACGAGTAGATGATACAACTG
	HindIII-BamH1 trfA E10	GCCCAAGCTTGGATCCTTATTCAGTTGTCTCTGGAAAATAACC
qRT-PCR probes and primers	*trfA* -102T FAM-TAMRA	AGCAACGAACAAAAGAAAAAGAAGCTCAAGGTT
	trfA -159R	CGTGCTGAAGACTTTTGACGTT
	trfA -70F	AGAAGGTGAAGATCAATTAGAAGAATTATTC
	HU-1708 FAM-TAMRA	CGTGAACGTGCTGCACGTAA
	HU-1687 F	GGTTTCGGTAACTTTGAGG
	HU-1747R	CAGTTTGAGGGTTACGACC
	spx - 294T FAM-TAMRA	TAAACGACTACAAGTTGGTTATAAT
	spx - 360R	CGTACGAACTTTTCTAGGTAAGAA
	spx - 263F	GCTTATTACGTCGTCCAATTATTTTA

[1] Underlined regions represent restriction enzyme sequences.