

Supplemental Material for Gaidenko *et al.*

Supplemental Tables S1, S2; Supplemental Figures S1, S2; Supplemental figure legends S1, S2.

Table S1. *Bacillus subtilis* strains

Strain	Genotype	Construction ¹
PB2	<i>trpC2</i>	168 Marburg strain
PB198	<i>amyE::ctc-lacZ trpC2</i>	(1)
PB1078	<i>rsbRBΔ2 rsbRCΔ1::ery rsbRDΔ2 amyE::ctc-lacZ trpC2</i>	(3)
PB1105	<i>rsbRA K143E rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5973 → PB1078 ²
PB1107	<i>rsbRA Q142A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5951 → PB1078 ²
PB1108	<i>rsbRA E148K rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5974 → PB1078 ²
PB1110	<i>rsbRA L149R rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5978 → PB1078 ²
PB1138	<i>rsbRA L146R rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5976 → PB1078 ²
PB1140	<i>rsbRA V139R rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG5977 → PB1078 ²
PB1141	<i>rsbRA E136K rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	(3)
PB1147	<i>rsbRA K143A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6004 → PB1078 ²
PB1148	<i>rsbRA E148A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6005 → PB1078 ²
PB1149	<i>rsbRA L149A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6006 → PB1078 ²
PB1151	<i>rsbRA Q142A rsbS S59A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6009 → PB1107 ²
PB1152	<i>rsbRA E148K rsbS S59A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6009 → PB1108 ²
PB1153	<i>rsbRA L146A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6017 → PB1078 ²
PB1154	<i>rsbRA V139A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6016 → PB1078 ²
PB1161	<i>rsbS S59A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	(3)
PB1166	<i>rsbRA L149D rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6020 → PB1078 ²
PB1179	<i>rsbRA K137A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6021 → PB1078 ²
PB1180	<i>rsbRA T138A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6022 → PB1078 ²
PB1182	<i>rsbRA Q142A amyE::ctc-lacZ trpC2</i>	pTG5951 → PB198 ²
PB1183	<i>rsbRA E148K amyE::ctc-lacZ trpC2</i>	pTG5974 → PB198 ²
PB1188	<i>rsbRA S140A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6023 → PB1078 ²
PB1189	<i>rsbRA L141A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6024 → PB1078 ²
PB1192	<i>rsbRA I144A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6025 → PB1078 ²
PB1193	<i>rsbRA Q147A rsbRBΔ2 rsbRDΔ2 rsbRCΔ1::ery amyE::ctc-lacZ trpC2</i>	pTG6026 → PB1078 ²

¹Arrow indicates transformation from donor to recipient

²Two-step allele replacement

Table S2. Plasmids used for strain construction

Plasmid	Alteration or relevant feature	Reference
pSS4332	Expresses I-SceI for two-step allele replacement	(2)
pTG5916	pUS19-based integrative plasmid. <i>NdeI</i> site converted to I-SceI	(3)
pTG5923	<i>rsbRA</i> in pTG5916	(3)
pTG5951	<i>rsbRA</i> Q142A in pTG5916 (CAA → GCA)	This study
pTG5973	<i>rsbRA</i> K143E in pTG5916 (AAA → GAA)	This study
pTG5974	<i>rsbRA</i> E148K in pTG5916 (GAG → AAG)	This study
pTG5976	<i>rsbRA</i> L146R in pTG5916 (CTG → CGA)	This study
pTG5977	<i>rsbRA</i> V139R in pTG5916 (GTA → CGA)	This study
pTG5978	<i>rsbRA</i> L149R in pTG5916 (CTG → CGA)	This study
pTG6004	<i>rsbRA</i> K143A in pTG5916 (AAA → GCA)	This study
pTG6005	<i>rsbRA</i> E148A in pTG5916 (GAG → GCG)	This study
pTG6006	<i>rsbRA</i> L149A in pTG5916 (CTG → GCG)	This study
pTG6009	<i>rsbS</i> S59A in pTG5916 (TCA → GCA)	(3)
pTG6016	<i>rsbRA</i> V139A in pTG5916 (GTA → GCA)	This study
pTG6017	<i>rsbRA</i> L146A in pTG5916 (CTG → GCG)	This study
pTG6020	<i>rsbRA</i> L149D in pTG5916 (CTG → GAC)	This study
pTG6021	<i>rsbRA</i> K137A in pTG5916 (AAA → GCA)	This study
pTG6022	<i>rsbRA</i> T138A in pTG5916 (ACA → GCA)	This study
pTG6023	<i>rsbRA</i> S140A in pTG5916 (TCC → GCC)	This study
pTG6024	<i>rsbRA</i> L141A in pTG5916 (CTG → GCG)	This study
pTG6025	<i>rsbRA</i> I144A in pTG5916 (ATC → GCC)	This study
pTG6026	<i>rsbRA</i> Q147A in pTG5916 (CAA → GCA)	This study

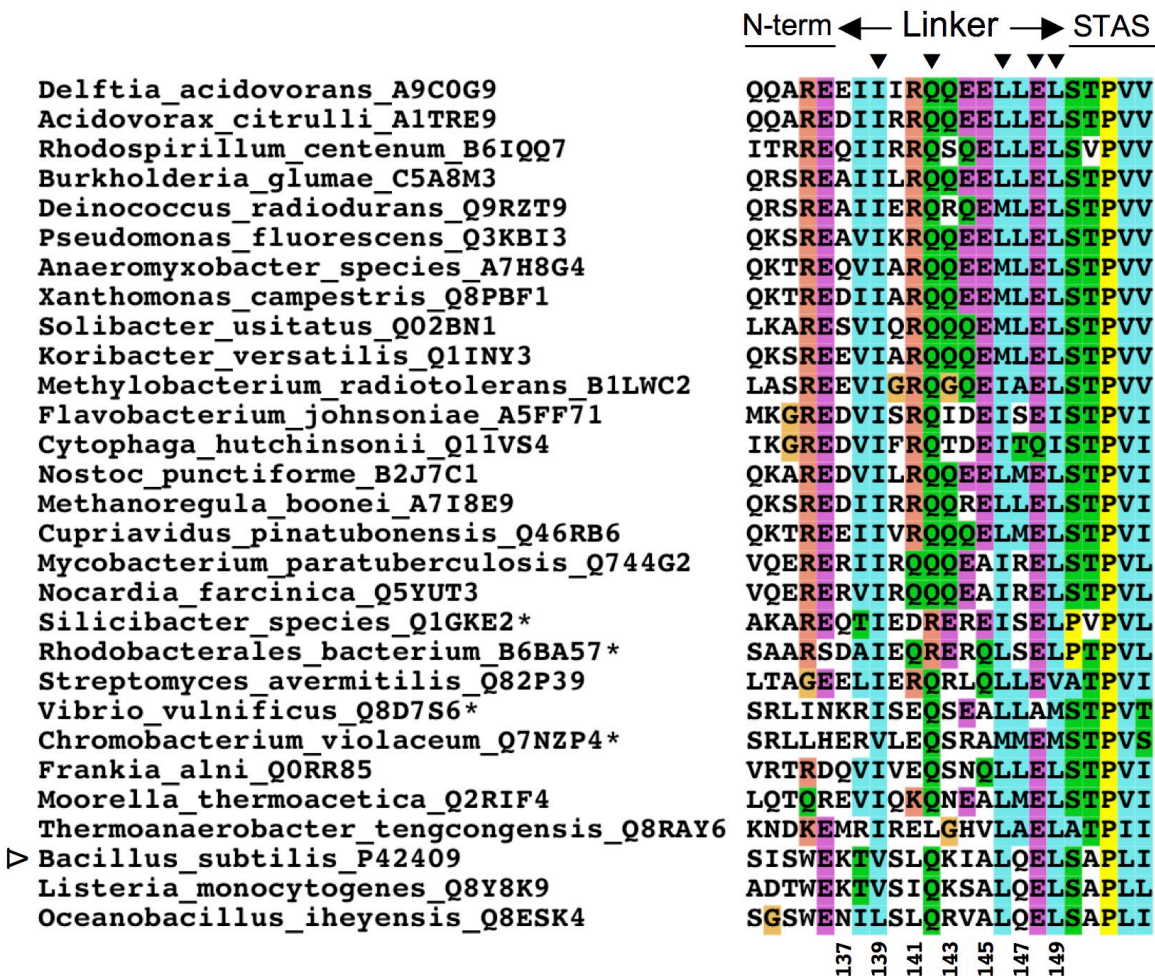


Fig. S1. Clustal W alignment of 29 linker regions from RST modules encoded by diverse microbial genomes. Sequence of each RsbRA homologue is labeled by genus_species and UniProt identifier; these represent one archaeal and seven bacterial phyla. According to the Conserved Domain Database (4), all listed homologues have N-terminal non-heme globin domains except the four protobacterial examples marked by the asterisk following their UniProt identifier; these four possess heme-binding globin domains. Labels above the alignment indicate five residues from the N-terminal globin domain, the 13-residue linker, and five residues from the C-terminal STAS (sulfate transporter and anti-sigma factor antagonist) domain; filled inverted triangles show five highly conserved linker positions. Numbers beneath the alignment designate residue number within *B. subtilis* RsbRA, whose sequence is marked by the open triangle on the left. Color scheme is standard Clustal W: blue for WLWIMFAC; green for TSNQ; magenta for DE; red for KR, orange for G; and yellow for P.

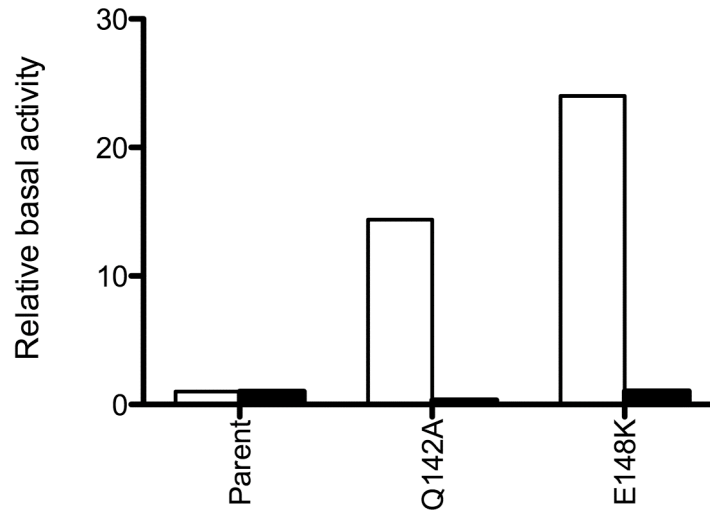


Fig. S2. Presence of all four RsbR co-antagonist paralogs masks the enhancing phenotype caused by linker substitutions in RsbRA. White bars show relative basal level in strains encoding only RsbRA, with data taken from Figure 3; black bars show relative basal activity in strains encoding a full co-antagonist complement: RsbRA, RB, RC and RD. Parent indicates wild type RsbRA, whereas Q142A and E148K indicate RsbRA substitution.

References

1. **Boylan, S. A., A. Rutherford, S. M. Thomas, and C. W. Price.** 1992. Activation of *Bacillus subtilis* transcription factor σ^B by a regulatory pathway responsive to stationary-phase signals. *J Bacteriol* **174**:3695-706.
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3. **Gaidenko, T. A., X. Bie, E. P. Baldwin, and C. W. Price.** 2011. Substitutions in the presumed sensing domain of the *Bacillus subtilis* stressosome affect its basal output but not response to environmental signals. *J Bacteriol* **193**:3588-97.
4. **Marchler-Bauer, A., et al.** 2009. CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res* **37**:D205-10.