Supplemental material for: Rap-protein paralogs of *Bacillus thuringiensis*: a multifunctional and redundant regulatory repertoire for the control of collective functions.

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Supplementary Figures.

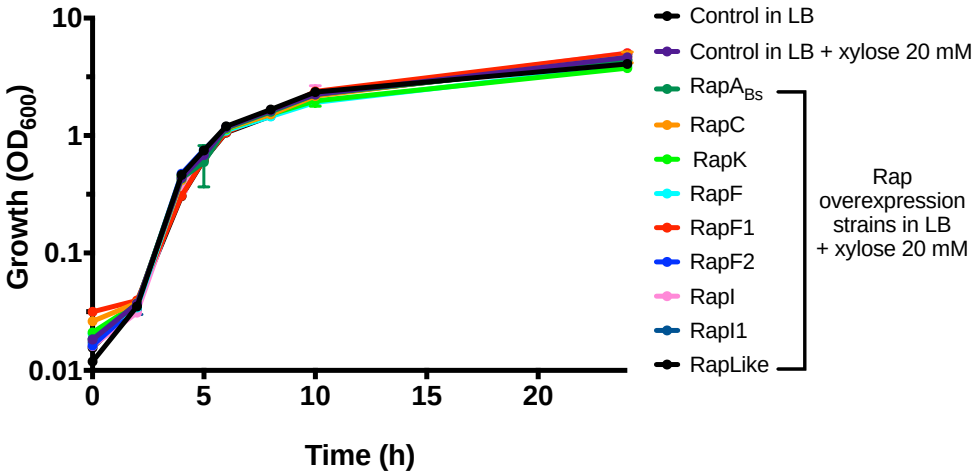

Figure S1. Growth curves of Rap-overexpression strains. Preinoculums of the control strain, and strains carrying plasmids for Rap overexpression, were washed and used to inoculate 30 ml of fresh media to a starting OD_{600} ≈0.03, in triplicate 125 ml flasks. Cultures were incubated at 30 ºC with shaking, following OD_{600} for 24 h. Each data point represents average of three replicates ± SD.

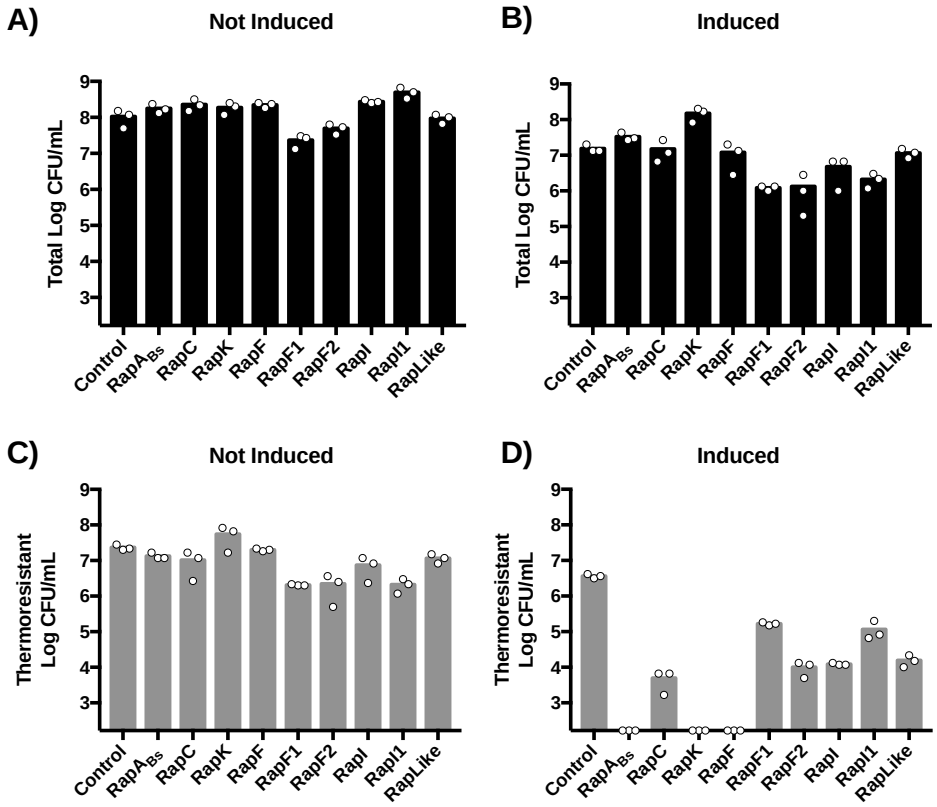

Figure S2. Effect of the induction of Rap overexpression in viability and sporulation of Bt8741. A-B) Total and C-D) thermoresistant CFU counts were obtained from triplicate 72 h cultures of control and Rap-overexpression strains, from LB with and without the addition of 20 mM xylose. Columns indicate average of three replicates; dots indicate individual data. Y-axis lower limit is adjusted to the limit of detection of the assay.

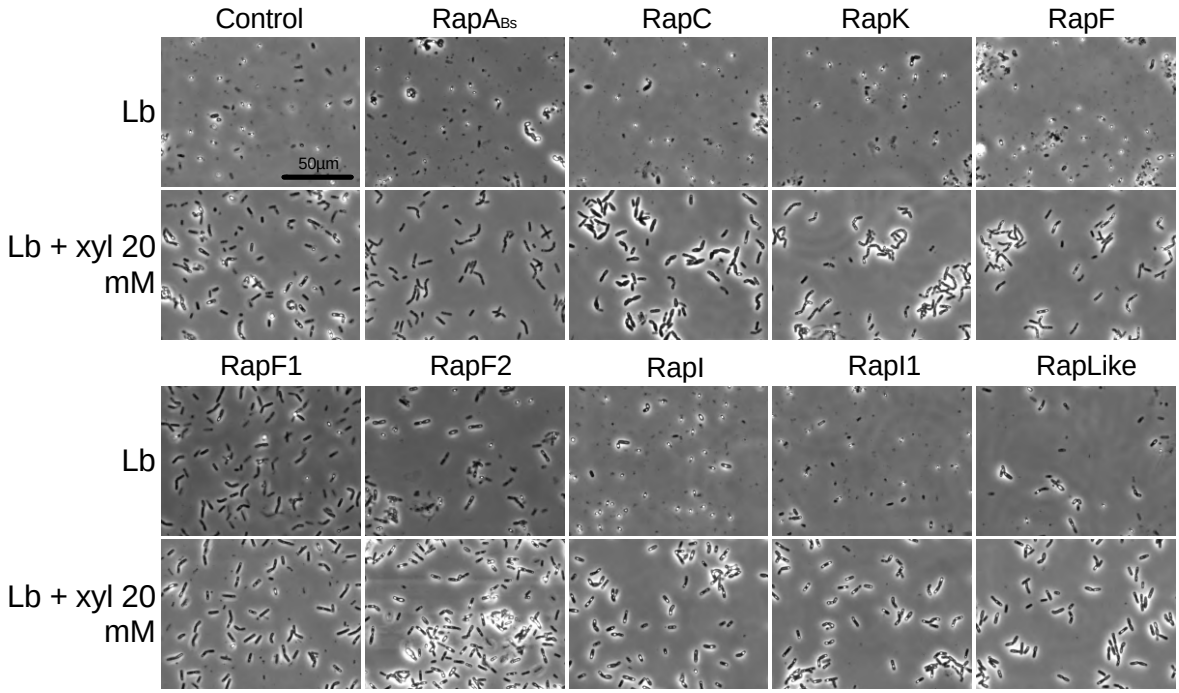

Figure S3. Cell morphology of control strain and Rap-overexpression strains. Samples were taken from induced cultures at 72 h. Phase contrast microscopy 63X + 1.8X magnification.

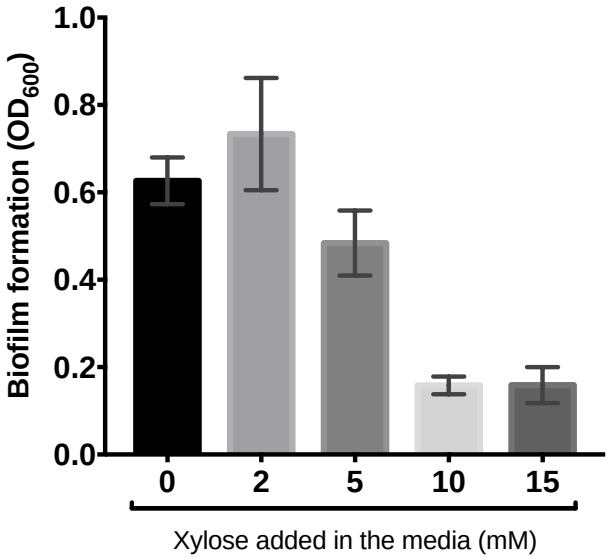

Figure S4. Biofilm formation of Bt8741 is inhibited by xylose addition. Three µl of preinoculum of the control strain, were used to inoculate 3 mL of nutrient broth with different concentrations of xylose. After 48 h, cells from the biofilm were recovered, suspended in 1.5 ml of PBS, and OD_{600} was measured. Columns indicate average of three replicates ± SD.

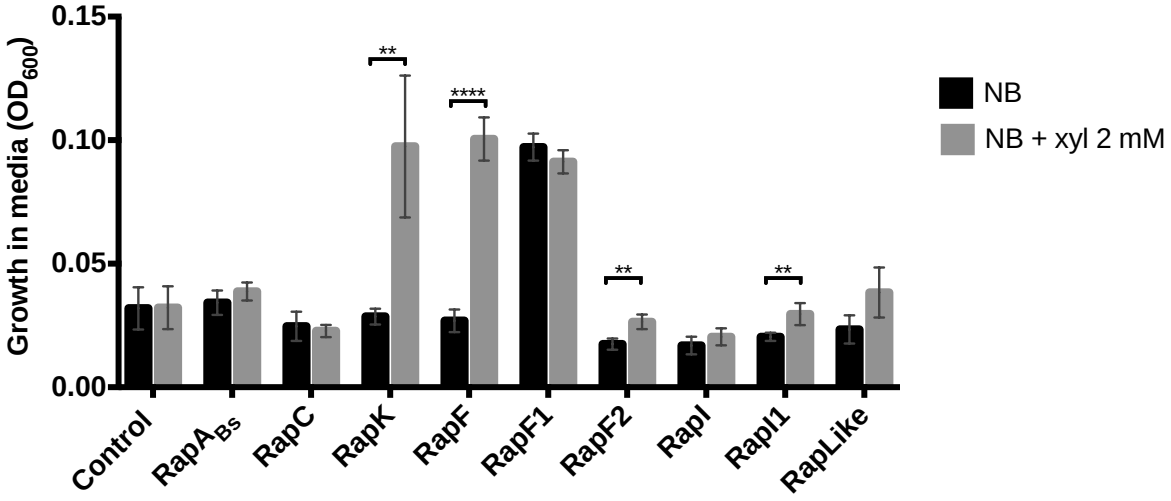

Figure S5. Planktonic growth of control and Rap-overexpression strains, during the assays for biofilm formation. OD_{600} was measured from the liquid media after 48 h. Columns indicate average of five individual measurements ± SD. NB, Nutrient Broth; **, $p<0.005$; ****, $p<0.0001$.

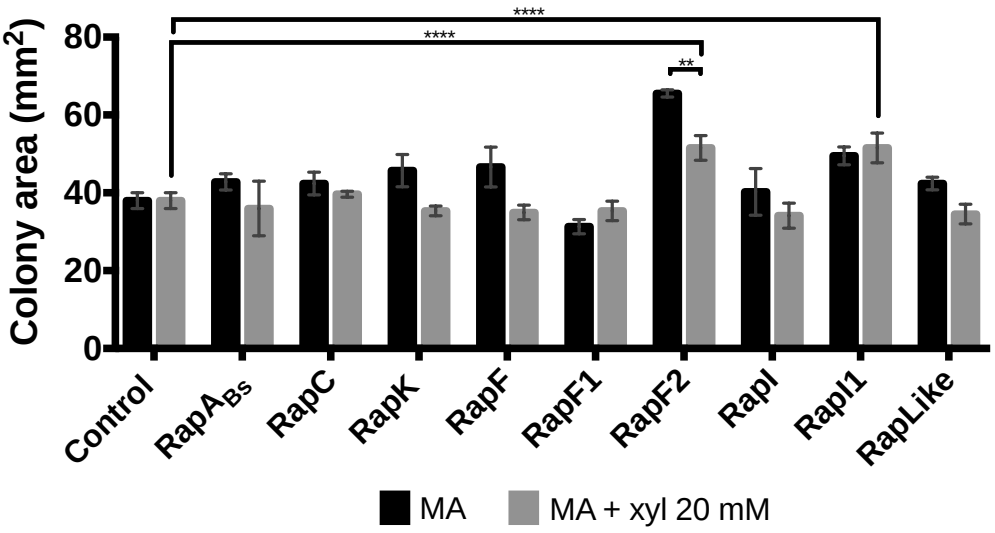

Figure S6. Colony area of Rap overexpression strains in milk agar after 24 h of incubation. Columns indicate average of triplicate measurements ± SD. MA, milk agar. **, $p<0.005$; ****, $p<0.0001$.

Supplementary Tables.

Table S1. Strains and plasmids used in this study

Strain	**Description**	**Reference**
Bacillus thuringiensis 8741	Wild type	(1)
Bacillus subtilis 168	Wild type	(2)
Escherichia coli TOP10	Strain used for construction and cloning of overexpression plasmids	(3)
Plasmid	**Description**	**Reference**
pHT315	Cloning vector for Bt ≈15 copies per cell	(4)
pHT315- P_{xylA}	Xylose inducible overexpression plasmid	This study
pHT315- P_{xylA}*'rapC*	Plasmid for overexpression of RapC from Bt8741	This study
pHT315- P_{xylA}*'rapK*	Plasmid for overexpression of RapK from Bt8741	This study
pHT315- P_{xylA}*'rapF*	Plasmid for overexpression of RapF from Bt8741	This study
pHT315- P_{xylA}*'rapF1*	Plasmid for overexpression of RapF1 from Bt8741	This study
pHT315- P_{xylA}*'rapF2*	Plasmid for overexpression of RapF2 from Bt8741	This study
pHT315- P_{xylA}*'rapI*	Plasmid for overexpression of RapI from Bt8741	This study
pHT315- P_{xylA}*'rapI1*	Plasmid for overexpression of RapI1 from Bt8741	This study
pHT315- P_{xylA}*'rapLike*	Plasmid for overexpression of RapLike from Bt8741	This study
pHT315- P_{xylA}*'rapA*	Plasmid for overexpression of RapA from Bs168	This study

Table S2. Oligonucleotides used in this study.

Name	Sequence (5' to 3')	Description
GG1	AAGAAGCTTCCATGTCACTATTGCTTCAG	Fwd *xylR*
GG2	CTGCTGCAGAGATTGAGCCATGTGATTTC	Rev P_{xylA}
GG3	CTGCTGCAGGACGTTCAAACAAAAAGTAATG	Fwd *rapK*
GG4	GTCGTCGACTTCCCATTAATGTTAGGATTG	Rev *rapK*
GG5	CTGCTGCAGCAACTAGGAAACGAACAAATTAC	Fwd *rapI*
GG6	GTCGTCGACTTCATCATTTCAATGACTCC	Rev *rapI*
GG7	CTGCTGCAGACAGCAACCAGTAATGAGAAG	Fwd *rapF*
GG8	GTCGTCGACGTTTTCTTCATTTTAATGCCTC	Rev *rapF*
GG9	CTGCTGCAGAACACTAAGTTTTTGACCCAAG	Fwd *rapC*
GG10	GTCGTCGACTTTTCATCACTGTAACGCTC	Rev *rapC*
GG11	CTGCTGCAGAGTGTACATGTAATAAAAAAGGAAG	Fwd *rapF1*
GG12	GTCGTCGACCAATTGAACTGCTGATGATC	Rev *rapF1*
GG13	CTGCTGCAGAACGTTCAACTACAAGGTAATG	Fwd *rapF2*
GG14	GTCGTCGACTATCATTTTAATGCCCCTTTC	Rev *rapF2*
GG15	CTGCTGCAGGGAGCAGATGTAGTAACGC	Fwd *rapI1*
GG16	GTCGTCGACATAGCCAAACGAAATTTCTTC	Rev *rapI1*
GG17	CTGCTGCAGTTATTAAAAGGGCATGAACAG	Fwd *raplike*
GG18	GTCGTCGACTCATTATCTTAGTGCCTCCTTAG	Rev *raplike*
GG19	CTGCTGCAGAGGATGAAGCAGACGATTC	Fwd *rapA*
GG20	GTCGTCGACAACAAACCTGACATCCATTTAG	Rev *rapA*
GG26	AACATAGTACATAGCGAATCTTC	Fwd P_{xylA}
DS16	CAGGCTTTACACTTTATGC	Fwd pHT315
DS17	CGATTAAGTTGGGTAACG	Rev pHT315

Underlined sequence indicates restriction site; Fwd, forward primer; Rev, reverse primer.

References

1. Rocha J, Flores V, Cabrera R, Soto-Guzmán A, Granados G, Juaristi E, Guarneros G, De La Torre M. 2012. Evolution and some functions of the NprR-NprRB quorum-sensing system in the *Bacillus cereus* group. Appl Microbiol Biotechnol 94:1069–1078.
2. Perego M, Hanstein C, Welsh KM, Djavakhishvili T, Glaser P, Hoch JA. 1994. Multiple protein-aspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in *B. subtilis*. Cell 79:1047–1055.
3. Maniatis T, Fritsch EF, Sambrook J. 1988. Molecular Cloning : a laboratory manual. 2nd edition. Cold Spring Harb ,New York.
4. Arantes O, Lereclus D. 1991. Construction of Cloning Vectors for *Bacillus thuringiensis*. Gene 108:115–119.