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A Small RNA Controls a Protein Regulator Involved in Antibiotic Resistance in *Staphylococcus aureus*.

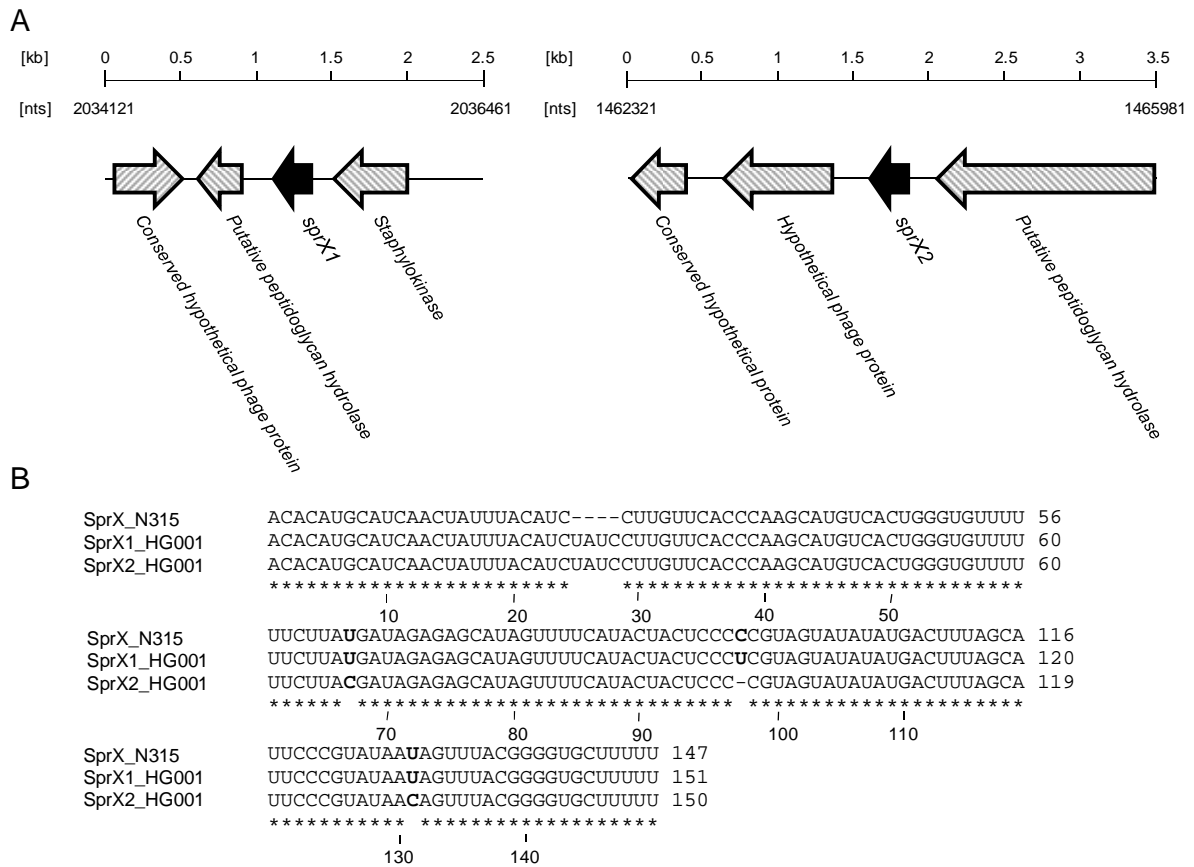
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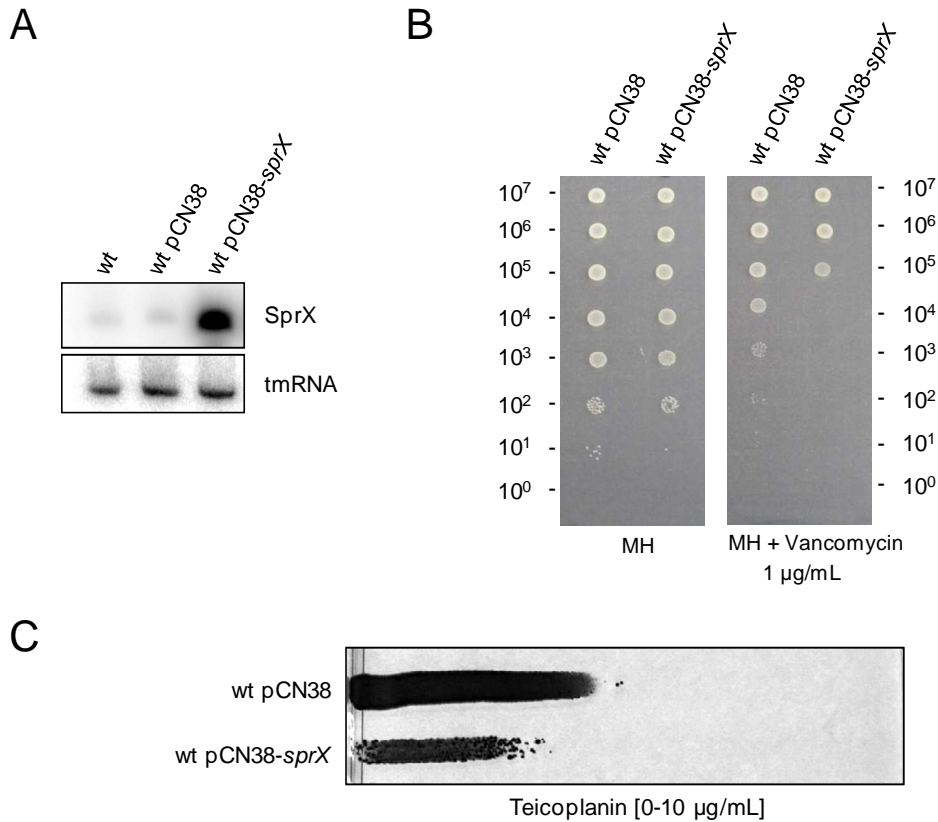
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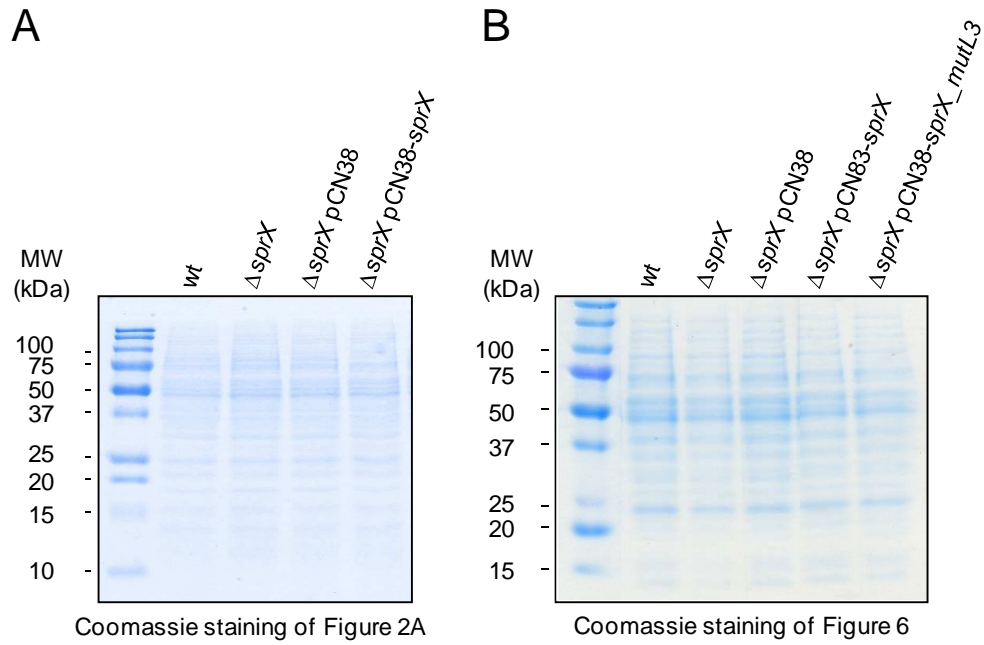
Supplementary data



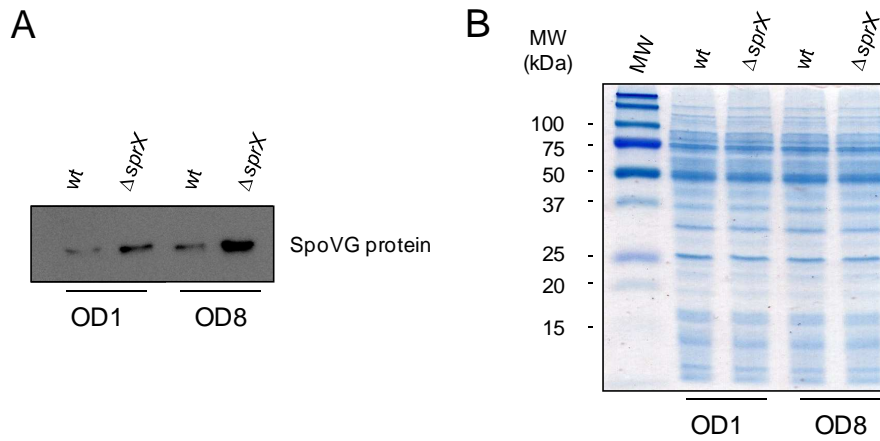
Supplementary Figure S1. Genomic location and sequence alignments of *sprX*. (A) Schematic representations of the genetic organization of the *S. aureus sprX1* and *sprX2* loci from strain HG001. The ORFs, *sprX* genes and nucleotide (nts) numbers correspond to those of strain HG001. (B) Alignments of the nucleotide sequences of SprX from strain N315 and the two SprX copies from strain HG001. Identical nucleotides in the sequences are indicated with asterisks. The nucleotide numbering is based on the SprX2 sequence.



Supplementary Figure S2. SprX modulates *S. aureus* glycopeptide resistance in VISA strain Mu50. (A) Upper panel: Northern blots showing SprX expression when grown until the late-exponential-phase in the Mu50 (wt) strain, Mu50 transformed by either pCN38 (wt pCN38) or by pCN38-*sprX* (wt pCN38-*sprX*). Lower panel: tmRNA used as a loading control. (B) Effect of SprX measured by agar plate assay in the presence of Teicoplanin. Overnight cultures were prepared in BHI of wt Mu50 strain transformed by either pCN38 (wt pCN38) or by pCN38-*sprX* (wt pCN38-*sprX*). Tenfold serial dilutions of cultures were deposited from top (most concentrated: 10⁷ bacteria) to bottom (10⁰ bacteria) on Mueller-Hinton (MH) plates and on MH plates supplemented with either Teicoplanin. (C) Evaluation of the levels of resistance of Mu50 transformed by pCN38 (wt pCN38) and wt Mu50 transformed by pCN38-*sprX* (wt pCN38-*sprX*) on gradient plate containing Teicoplanin.

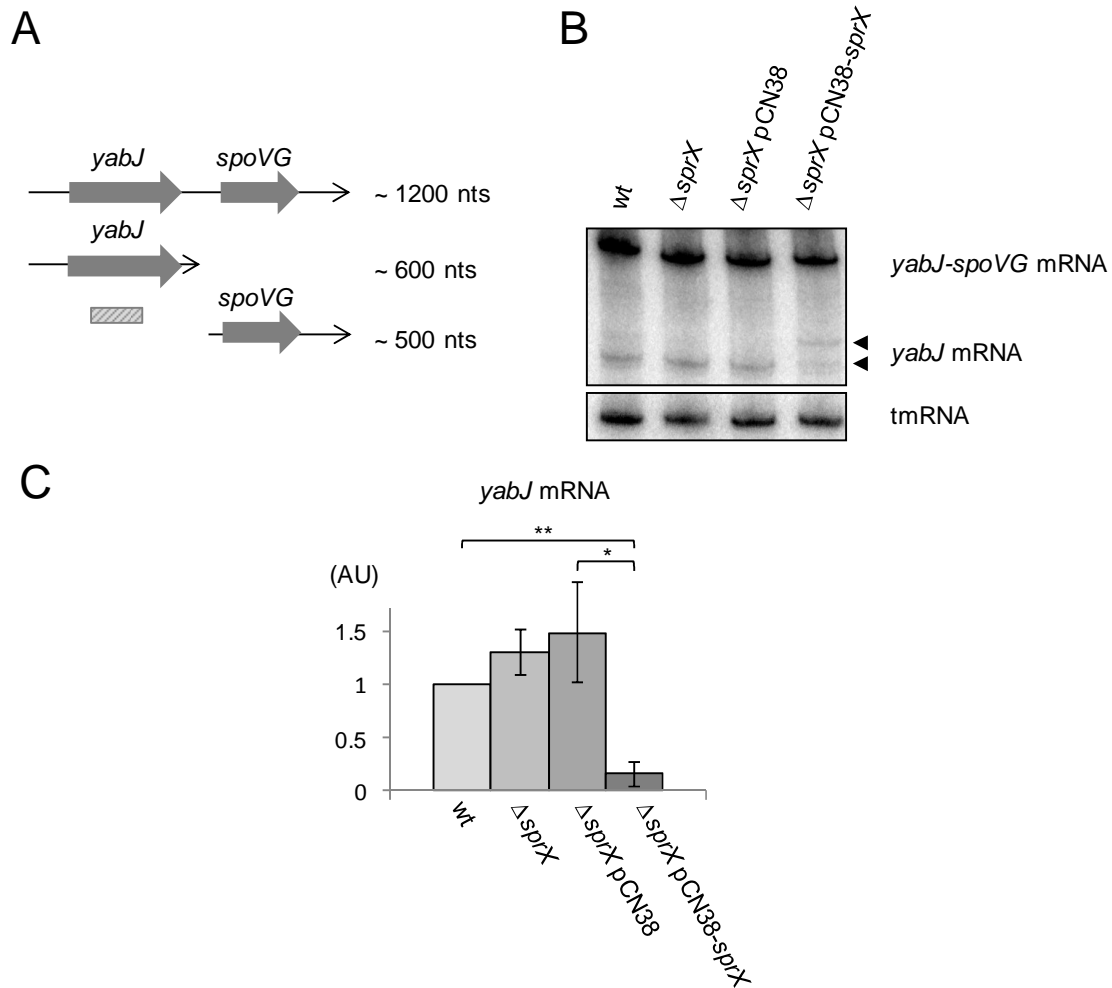


Supplementary Figure S3. Coomassie stained gel loading controls. (A) Samples presented in Figure 2A, panel A. (B) Samples presented in Figure 6. Gels indicated that identical protein amounts were loaded for the HG001 wt (wt) and HG001 $\Delta sprX$ ($\Delta sprX$) and in HG001 $\Delta sprX$ transformed by either pCN38 ($\Delta sprX$ pCN38), pCN38-*sprX* ($\Delta sprX$ pCN38-*sprX*) or pCN38-*sprX*_mutL3 ($\Delta sprX$ pCN38-*sprX*_mutL3).



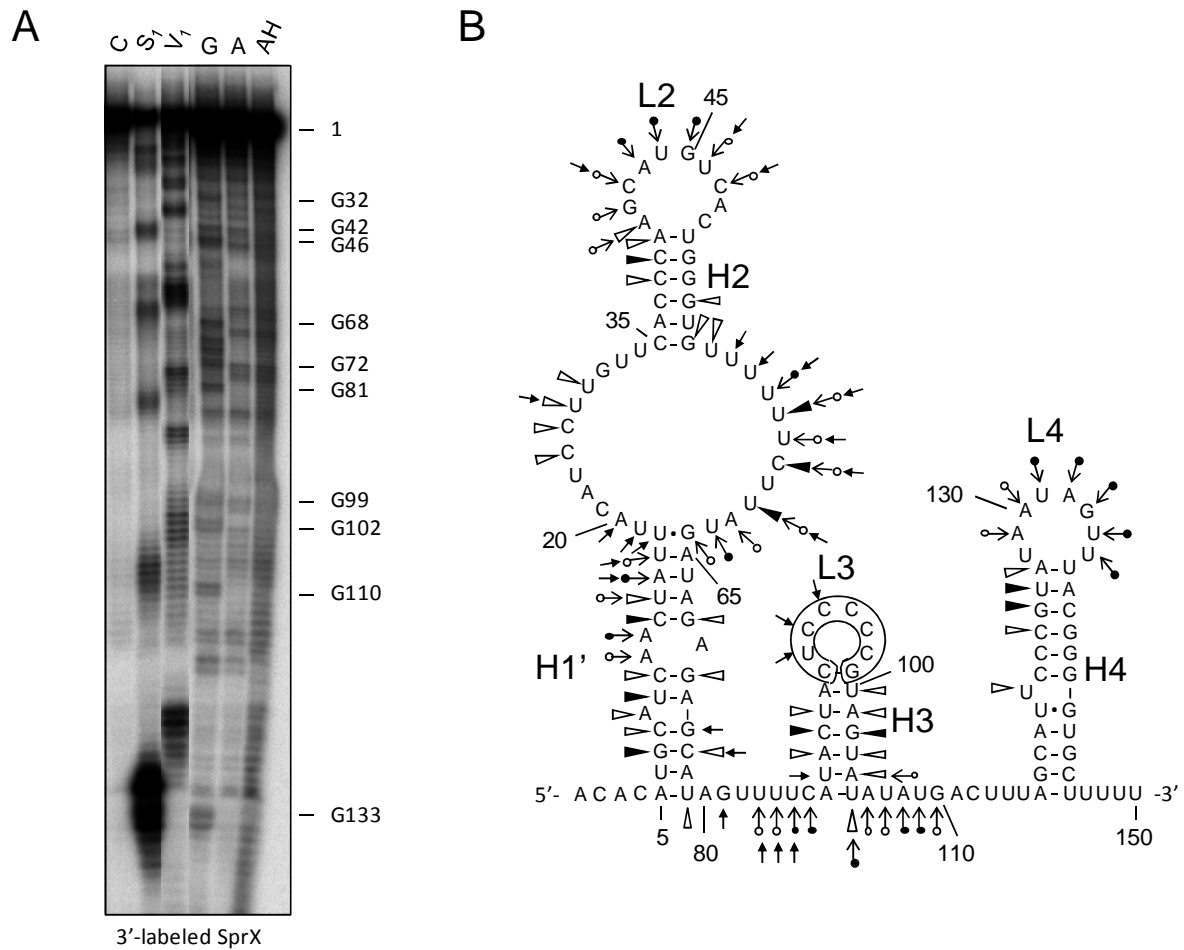
Supplementary Figure S4. SprX inhibits SpoVG protein expression during growth. (A)

Western blot of SpoVG protein levels when grown until the early- (OD₆₀₀ 1) and late-exponential (OD₆₀₀ 8) phases of growth in the wt HG001 strain (wt) and HG001 $\Delta sprX$ ($\Delta sprX$). (B) Coomassie stained gel loading controls of samples presented on panel A.

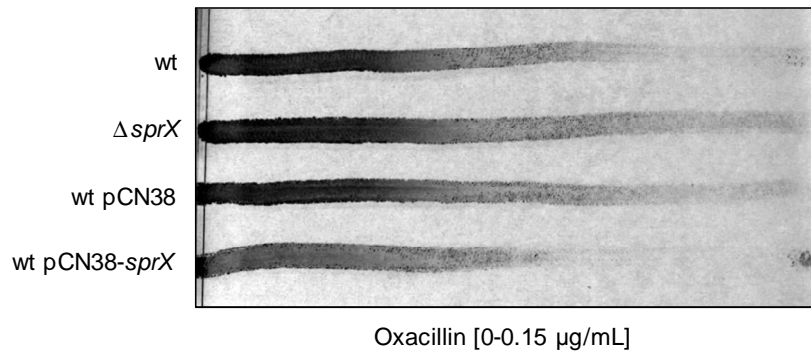


Supplementary Figure S5. Effect of SprX on *yabJ* and *yabJ-spoVG* mRNAs expression levels. (A) Schematic representation of *yabJ-spoVG* mRNA. ORFs, mRNA lengths and the probe used for detection by Northern blots of *yabJ* and *yabJ-spoVG* mRNAs are indicated. nts, nucleotides. (B) Northern blots were used to detect expression levels of *yabJ* and the *yabJ-spoVG* mRNAs in strain HG001 wt (wt) and HG001 Δ *sprX* (Δ *sprX*) and in HG001 Δ *sprX* transformed by either pCN38 (Δ *sprX* pCN38) or pCN38-*sprX* (Δ *sprX* pCN38-*sprX*). These were grown until 7.5 hours of growth. Probe against tmRNA was used as a loading control (probes are listed in Table II). (C) Northern blot quantification of the levels of *yabJ* and *yabJ-spoVG* mRNAs of the strains from panel C. mRNA expression levels were calculated relative to the value measured for the wt strain. The error bars are the mean values derived from three independent experiments with independent RNA purifications. The tmRNA was used for

normalization. The value of the mRNA levels in wild-type strain was normalized to 1. AU, arbitrary units. Difference in expression was measured by student's T-test, *p-value < 0.05, **p < 0.01.



Supplementary Figure S6. An alternative conformation for SprX (strain N315) was proposed based on the probing data. (A) 3'-labeled SprX was probed by RNase V₁ (which cleaves double-strands or stacked nucleotides) and by nuclease S₁ and lead acetate (which both cleave the accessible single-strands). Lanes are as follows: C, control; S₁, Nuclease S₁; V₁, RNase V₁; Pb, lead-induced cleavages; G, RNase T₂; A, RNase U₂; and AH, alkaline ladders. SprX numbering is shown on the right. (B) Secondary structure of SprX from strain N315 based on probing data. The nucleotide numbering corresponds to SprX2 from HG001 (Supplementary Figure S1). The mutated nucleotides in SprX_mutL3 are outlined on the SprX secondary structure model. Enzymatic cleavages are as follows: moderate (o→) and strong (•→) S₁ Nuclease cleavages; (→) lead(II)-cleavages; moderate (⇨) and strong (⇩) RNase V₁ cleavages.



Supplementary Figure S7. SprX affects the Oxacillin sensibility of *S. aureus* strain HG001. Evaluation of the levels of resistance of the wt HG001 strain (wt); HG001 $\Delta sprX$ ($\Delta sprX$); wt HG001 transformed by pCN38 (wt pCN38); and wt HG001 transformed by pCN38-*sprX* (wt pCN38-*sprX*) on a gradient plate containing Oxacillin.

Table S1. Strains and plasmids used in this study.

Strains	Relevant characteristics	References
<i>E. coli</i> strains		
DH5a	F ⁺ ϕ 80d <i>lacZ</i> Δ M15 <i>D(lacZA-argF)U169 deoR recA1 endA1 hsdR17 (rK-mK-)</i> <i>phoA supE44 l- thi-1 gyrA96 relA1</i>	Invitrogen
BL21 (D3)	F ⁺ <i>ompT gal dcm lon hsdS_B (r_B- m_B-)</i> (DE3)	Novagen
<i>S. aureus</i> strains		
HG001	<i>rsbU</i> restored strain 8325, lysogenic for phages ϕ 11, ϕ 12, and ϕ 13	(1)
HG001 Δ <i>sprX</i>	HG001 deleted for <i>sprX1</i> and <i>sprX2</i> genes	This study
HG001 Δ <i>yabJ-spoVG::erm</i>	HG001 deleted for <i>yabJ-spoVG::erm(B)</i> ; Emr	This study
RN4220	Restriction-defective derivative of 8325-4	(2)
RN4220 Δ <i>yabJ-spoVG::erm</i>	RN4220 deleted for <i>yabJ-spoVG::erm(B)</i> ; Emr ^f	(3)
Mu50	VISA clinical isolate	(4)
Plasmids		
pCN38	Low-copy-number shuttle vector with Amp ^r in <i>E. coli</i> and Cm ^r in <i>S. aureus</i>	(5)
pCN38- <i>sprX</i>	pCN38 with <i>sprX</i> copy 2 of HG001 under the control of its endogenous promoter	This study
pCN38- <i>sprX_mutL3</i>	pCN38 with mutated C-rich loop L3 of <i>sprX2</i> under the control of its endogenous promoter	This study
pBT2	Low-copy-number shuttle vector with Ampr in <i>E. coli</i> and temperature sensitive replication with Cmr in <i>S. aureus</i>	(6)
pBT2 Δ <i>sprX1</i>	pBT2 vector containing the 1028-bp upstream genomic sequence of <i>sprX</i> copy 1 of HG001 and its 981-bp downstream sequence	This study
pBT2 Δ <i>sprX2</i>	pBT2 vector containing the 1050-bp upstream genomic sequence of <i>sprX</i> copy 2 of HG001 and its 948-bp downstream sequence	This study
pET-28a(+)	Expression vector; Kmr	Novagen
pSTM33	pET-28a(+) with 0.33-kb fragment covering <i>spoVG</i> ; Kmr	(3)

a Abbreviations: Ampr, ampicillin resistant; Emr, erythromycin resistant; Cmr : Chloramphenicol resistant; Kmr, kanamycin resistant.

Table S2. Primers used in this study.

DNAs	Sequences	Purposes
anti-C2	TACGGGAATGCTAAAGTCAT	SprX Northern
oSTM29	GCGTCGACTTATTGCAAATGTATTACATCGC	<i>yabJ-spoVG</i> Northern
oSTM30	CTAAATAA AACAGAGAGA TATATACTATAGG	
5S	CGTAAGTTCGACTACCATCG	5S Northern
tmRNA	ACACGCTTAATGAGCTCGGG	tmRNA Northern
5'PSTI_sprX2	TTAAATCTGCAGTATTTACTTAGAATAAAAAATTTTGC	pCN38- <i>sprX2</i>
3'EcoRI_sprX2	CAAAGGAATTCATTTCCACCTCTTTTAACACA	
mut for	AGCATAGTTTTCATACTAGAGTAAGCTAGTATATATGACTTTAG	pCN38- <i>sprX_mutL3</i>
mut rev	CTAAAGTCATATATACTAGCTTACTCTAGTATGAAAACATGCT	
T7SprXfor	TAATACGACTCACTATAGGGACACATGCATCAACTATTTAC	<i>sprX-N315</i> and <i>sprX1</i> transcription
T7SprXRev2A	AAAGCACCCCGTAAACTATTAT	
T7SprXRev2B	AAAGCACCCCGTAAACTGTTAT	<i>sprX2</i> transcription
T7yabJ-spoVGfor	TAATACGACTCACTATAGGGGTAGAATCAAAAGAAGTTAAAC	<i>yabJ-spoVG</i> transcription
T7yabJ-spoVGrev	AATACGAGCATCATTCAAACGCA	
T7spoVGfor	TAATACGACTCACTATAGGGGCTCACTACATGAAAGTGACA	<i>spoVG</i> transcription
fragSpoVGfor	TAATACGACTCACTATAGGGAATTATAATTTTCGATTAATA	<i>yabJ-spoVG₁₆₇</i> transcription
fragSpoVGrev	AAAGCTTCATCTAATGTAATGG	
Toeprint spoVG	CAATTACACGTAAATCATGAAT	<i>spoVG</i> toeprint
Toeprint yabJ	GTCCATCAATATTCAATGGAA	<i>yabJ</i> toeprint
sprXD1	ATCGGATATCGGATCCATAATGAGTGGATATGGCATGA	<i>sprX1</i> deletion
sprXD6	ATCGTAAGCTTCTGCAGTCCTACAAATGAAGAAATAGAATC	
sprXD7	CTTAACAGGCTATATAGTTCACTACTTTTTTCATCCTAACTGATTTTC	
sprXD8	GAAATCAGTTAGGATGAAAAAGTAGTGAACCTATATAGCCTGTTA AG	
2sprXD1	TTACGAAGCTTGGATCCATCCGTAATATATAACACCAAAT	<i>sprX2</i> deletion
2sprXD4	TTACGCTGCAGGAATTCTGTTGATGAGATATCAATGTAA	
2sprXD2	ACTATATAAAAGTAAGTATAACATACGAAGTACCTGCCAGTTAC GCA	
2sprXD3	TGCGTAACTGGCAGGTACTTCGTATGTTATACTTACTTTTATATAG T	

Table S3. Summary of the data collected by the DiGE analysis. 2D-DiGE performed on strain HG001 wt versus isogenic HG001 $\Delta sprX$ strain. Cultures were grown until the late exponential phase, when total proteins were extracted, labeled with different cyanine-dyes, and separated onto pH 4-7 acrylamide gels. The ratios correspond to the quantification obtained for the HG001 $\Delta sprX$ versus the wt strain (the data were collected in four different experiments).

protein name	Fold changes (HG001 $\Delta sprX$ /HG001 wt)
HYP protein SAV0170	1.4
UPF0356 protein USA300HOU_1032	1.3
Putative septation protein SpoVG	1.4
MerR family transcriptional regulator	1.5
Transketolase	1.4

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