Supplemental material

Control of natural transformation in salivarius streptococci through specific degradation of σ^X by the MecA-ClpCP protease complex

Astrid Wahl, Florence Servais, Anne-Sophie Drucbert, Catherine Foulon, Laetitia Fontaine, and Pascal Hols

Table S1. Bacterial strains and plasmids used in this study.

Table S2. Primers used in this study.

Table S3. Plasmids used for B2H in this study.

Fig. S1. Multiple sequence alignment of a selected set of MecA-like proteins from streptococci and MecA of *B. subtilis*.

Fig. S2. Predicted structure and protein sequence conservation of σ^X in streptococci.

Fig. S3. *In vitro* degradation of MecA and σ^X.

Fig. S4. MecA-σ^X cross-interactions evaluated by B2H on MacConkey indicator plates with cognate pairs of *S. thermophilus* LMD-9, *S. mutans* UA159, and *S. pneumoniae* R6.

TABLE S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristic(s)[a]	Source or reference
E. coli		
BTH101	F', *cya-99 araD139 galE15 galK16 rpsL1* (STr[r]) *hsdR2 mcrA1 mcrB1*	(1)
TG1	K-12 *supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5, (r$_K^-$m$_K^-$)*	(2)
S. thermophilus		
LMD-9	Wild type	ATCC[b]
CB007	LMD-9 *blpD-blpX*::P$_{comGA}$-*luxAB*	(3)
CB0072	CB007 *mecA::lox72*	(3)
CB0053	LMD-9 *comX::strep*	(3)
Plasmids		
pBADhisA	Ap[r], ColE1 replication origin, contains the arabinose-inducible promoter P$_{BAD}$	Invitrogen
pBADhisA-ComX	pBADhisA derivative containing σ^X fused to an N-terminal 6His tag	This study
pBAD-ComX-Strep	pBADhisA derivative containing σ^X fused to a C-terminal StreptagII	This study
pBADhisA-MecA	pBADhisA derivative containing MecA fused to an N-terminal 6His tag	This study
pBADhisA-ClpC	pBADhisA derivative containing ClpC fused to an N-terminal 6His tag	This study
pBADhisA-ClpP	pBADhisA derivative containing ClpP fused to an N-terminal 6His tag	This study
pBADhisA-ClpE	pBADhisA derivative containing ClpE fused to an N-terminal 6His tag	This study
pGIUD0855ery	Ap[r], Em[r]; pUC18ery derivative. This plasmid was constructed to assess the natural transformation rate of *S. thermophilus* strains	(4)
pUT18	Ap[r]; pUC19 derivative containing the T18 fragment of CyaA under the control of the P$_{lac}$ promoter for in-frame X-T18 fusions	(5)
pUT18C	Ap[r]; pUC19 derivative containing the T18 fragment under the control of the P$_{lac}$ promoter for in-frame T18-X fusions	(5)
pKNT25	Km[r]; pSU40 derivative encoding the T25 fragment of CyaA under the control of the P$_{lac}$ promoter for in-frame X-T25 fusions	(6)
pKT25	Km[r]; pSU40 derivative encoding the T25 fragment of CyaA under the control of the P$_{lac}$ promoter for in-frame T25-X fusions	(6)

[a] Km[r], Em[r], and Ap[r]: kanamycin, erythromycin, and ampicillin resistance, respectively.
[b] ATCC, American Type Culture Collection.

TABLE S2. Primers used in this study

Primer	Sequence (5' to 3') [a]	Target
Primers used for the construction of over-expression plasmids (pBADhisA derivatives)		
aw14-mecAatgXbaI	CG<u>TCTAGA</u>ATGGAAATGAAACAAATAAGC	*mecA*
BD-mecAtermKpnI	CG<u>CGGTACC</u>CGGCTCCATTTCGTTGCTTGTAATAC	*mecA*
aw13-comXatgXbaI	GC<u>TCTAGA</u>ATGGAACAAGAAGTTTTTGTT	*comX*
BD-comXtermKpnI	GC<u>GGTACC</u>CGGTCTTCTTCATTACATGGATCAAAGTC	*comX*
aw15-clpCatgXbaI	CGG<u>TCTAGA</u>ATGACGATATATTCAAGAAAA	*clpC*
aw2-clpCtermKpnI	AA<u>GGTACC</u>CGCACTACTGTAAAGGTTAATTT	*clpC*
aw16-clpEatgXbaI	TG<u>TCTAGA</u>ATGCTCTGCCAAAACTGTAAC	*clpE*
aw4-clpEtermKpnI	TA<u>GGTACC</u>CGGTTGACTTCTTTTAATGCTTC	*clpE*
aw26-ClpPatgXbaI	CGC<u>TCTAGA</u>ATGATTCCGGTAGTTATTGAA	*clpP*
aw27-ClpPtermHindIII	GG<u>AAGCTT</u>TTTTAATTGGTTGTTGGTCAT	*clpP*
aw17-comX_STREPatg	CG<u>CCATGG</u>AACAAGAAGTTTTTGTT	*comX::strep*
aw18-comX_STREPterm	TTA<u>GGTACC</u>TCATTTCTCGAACTGCGGGTG	*comX::strep*
Primers used for the construction of B2H plasmids		
aw44-mecA$_{C121}$XbaI	CGG<u>TCTAGA</u>GAAAGAGGTTGATGAGACTAT	*mecA$_{C121}$*
aw46-comX$_{C65}$XbaI	AGC<u>TCTAGA</u>GCCCAATAAGGAGCTAGATATG	*comX$_{C65}$*
aw48-comXN25LKpnI	AG<u>GGTACC</u>ATACCACTAGCACTAAAGTATTGCCTAAA AGCCTT	*comX$_{N25}$*
aw49-comXN50LKpnI	GA<u>GGTACC</u>ATACCACTAGCACTAGGAAACTTTTTTAA AAGCTG	*comX$_{N1-50}$*
aw50-comXN75LKpnI	GA<u>GGTACC</u>ATACCACTAGCACTCACTTCATCATTAAG TCGATT	*comX$_{N1-75}$*
aw51-comXN100LKpnI	ATGGTACCATACCACTAGCAGCAATACAAAAGGCAAT ATCTGA	*comX$_{N1-100}$*
aw62-comXN58LKpnI	AT<u>GGTACC</u>ATACCACTAGCAGCCTTATCATCATCTTTC TCTAA	*comX$_{N1-58}$*
aw63-comXN68LKpnI	TA<u>GGTACC</u>ATACCACTAGCAGCCCTAAACTTAGTTTT AAAGTA	*comX$_{N1-68}$*
aw66-XbaIcomXN50	GA<u>TCTAGA</u>GGATTTAGAGAAAGATGATGAT	*comX$_{N50-75}$*
aw52-mecAL70LKpnI	CC<u>GGTACC</u>CGAATAGTTTCCTCAGCCACT	*mecA$_{Li70}$*
aw53-mecAL70LXbaI	AT<u>TCTAGA</u>GAGTGCTAGTGGAGATCTTAAGGAAGACC TTGAT	*mecA$_{Li70}$*
aw54-mecAN79LKpnI	AT<u>GGTACC</u>CATCCACTAGCACTATCTGATTTAGTCAC AAAAAC	*mecA$_{N1-79}$*
aw55-mecAC100LXbaI	AT<u>TCTAGA</u>AGTGCTAGTGGATGAAGATTATACTCACT ATGT	*mecA$_{C100}$*
aw73-comXspnAtgXbaI	GC<u>TCTAGA</u>GATGATTAAAGAATTGTATGAAGAAGTC	*comX$_{SPN}$*
aw74-comXspnTerKpnI	GC<u>GGTACC</u>GCATGGGTACGGATAGTAAACTC	*comX$_{SPN}$*
aw86-mecAspnAtgBamHI	GC<u>GGATCC</u>GATGAAAATGAAACAAATTAGT	*mecA$_{SPN}$*
aw76-mecAspnTerKpnI	GC<u>GGTACC</u>GCGCCGATTTTTTGCAGATTGAG	*mecA$_{SPN}$*
aw77-comXmutAtgXbaI	GC<u>TCTAGA</u>GATGGAAGAAGATTTTGAAATTGTT	*comX$_{MUT}$*
aw78-comXmutTerKpnI	GC<u>GGTACC</u>GCTTTTTCCTTAAAATCACTTAATTTTTTA C	*comX$_{MUT}$*
aw79-mecAmutTerKpnI	GC<u>GGTACC</u>GCTCCAATCATTTGTAATTCTTGC	*mecA$_{MUT}$*

[a] Restriction sites introduced in the primers are underlined.

TABLE S3. Plasmids used for B2H in this study

| Plasmid	Characteristics [a]	Source or reference
pKNT25-ClpC	P_{lac}- $clpC$-$cyaA^{1-732}$ kan, ClpC-T25 fusion protein	Boutry et al., 2012
pUT18-MecA	P_{lac}-$mecA$-$cyaA^{675-1197}$ bla, MecA-T18 fusion protein	Boutry et al., 2012
pUT18C-MecA	P_{lac}-$cyaA^{675-1197}$-$mecA$ bla,T18-MecA fusion protein	Boutry et al., 2012
pUT18-MecA$_{N1-79}$	P_{lac}-$mecA_{N1-79}$-$cyaA^{675-1197}$ bla, MecA$_{N1-79}$-T18 fusion protein	This study
pUT18C-MecA$_{Li70}$	P_{lac}- $cyaA^{675-1197}$- $mecA_{Li70}$ bla, T18-MecA$_{Li70}$ fusion protein	This study
pUT18C-MecA$_{C100}$	P_{lac}- $cyaA^{675-1197}$-$mecA_{C100}$ bla, T18-MecA$_{C100}$ fusion protein	This study
pUT18C-MecA$_{N1-103}$	P_{lac}-$cyaA^{675-1197}$-$mecA_{N1-103}$ bla, T18-MecA$_{N1-103}$ fusion protein	This study
pUT18C-MecA$_{C121}$	P_{lac}-$cyaA^{675-1197}$-$mecA_{C121}$ bla, T18-MecA$_{C121}$ fusion protein	This study
pKNT25-ComX	P_{lac}-$comX$-$cyaA^{1-732}$ kan, σ^X-T25 fusion protein	(3)
pKT25-ComX$_{C65}$	P_{lac}-$cyaA^{1-732}$-$comX_{C65}$ kan, T25- σ^X_{C65} fusion protein	This study
pKNT25-ComX$_{N1-100}$	P_{lac}-$comX_{N1-100}$-$cyaA^{1-732}$ kan, σ^X_{N1-100}-T25 fusion protein	This study
pKNT25-ComX$_{N1-75}$	P_{lac}-$comX_{N1-75}$-$cyaA^{1-732}$ kan, σ^X_{N1-75}-T25 fusion protein	This study
pKNT25-ComX$_{N1-68}$	P_{lac}-$comX_{N1-68}$-$cyaA^{1-732}$ kan, σ^X_{N1-68}-T25 fusion protein	This study
pKNT25-ComX$_{N1-58}$	P_{lac}-$comX_{N1-58}$-$cyaA^{1-732}$ kan, σ^X_{N1-58}-T25 fusion protein	This study
pKNT25-ComX$_{N1-50}$	P_{lac}-$comX_{N1-50}$-$cyaA^{1-732}$ kan, σ^X_{N1-50}-T25 fusion protein	This study
pKNT25-ComX$_{N50-75}$	P_{lac}-$comX_{N50-75}$-$cyaA^{1-732}$ kan, σ^X_{N50-75}-T25 fusion protein	This study
pKNT25-ComX$_{MUT}$	P_{lac}-$comX$-$cyaA^{1-732}$ kan, σ^X_{MUT}-T25 fusion protein	This study
pKNT25-ComX$_{SPN}$	P_{lac}-$comX$-$cyaA^{1-732}$ kan, σ^X_{SPN}-T25 fusion protein	This study
pUT18-MecA$_{MUT}$	P_{lac}-$mecA$-$cyaA^{675-1197}$ bla, MecA$_{MUT}$-T18 fusion protein	This study
pUT18-MecA$_{SPN}$	P_{lac}-$mecA$-$cyaA^{675-1197}$ bla, MecA$_{SPN}$-T18 fusion protein	This study

[a] kan, bla: kanamycin, ampicillin resistance. The N or C followed by a number as index indicates the N- or C-terminus and the number of amino acids from the beginning or the end of the protein, respectively; i.e. MecAN1-79 stands for the first 79 aa, MecALi70 for the linker domain (composed of 70 aa) and MecAC100 for the last 100 aa of MecA. MUT and SPN in the index stand for *S. mutans* UA159 and *S. pneumoniae* R6, respectively.
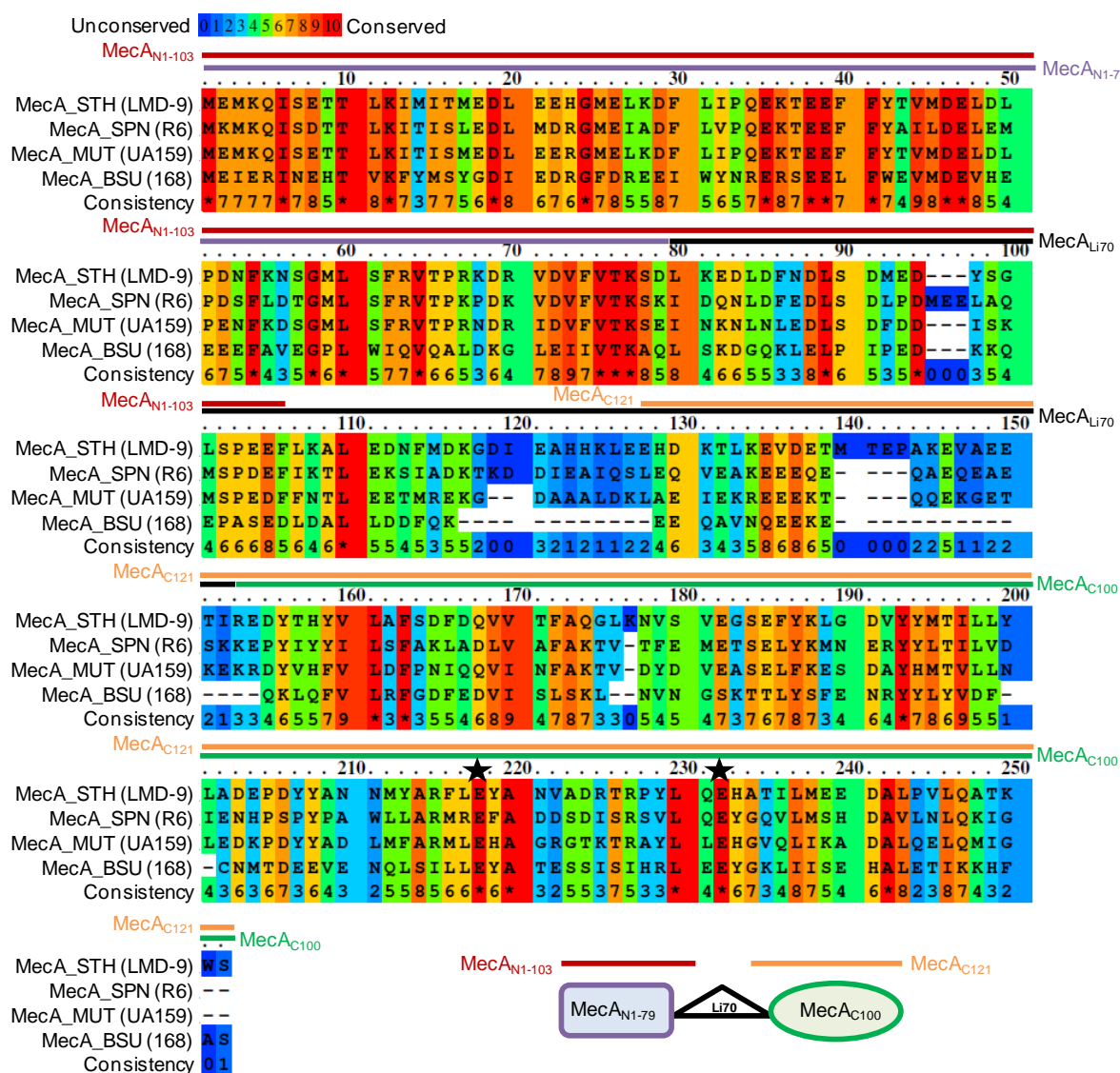
Fig. S1. Multiple sequence alignment of a selected set of MecA-like proteins from streptococci and MecA of *B. subtilis*. Sequence alignment was performed with the PRALINE package (http://www.ibi.vu.nl/programs/pralinewww/). Conservation is represented by a color code indicated on the top of the alignment where dark blue and red represent the less and most conserved residues, respectively. Similarity scores are indicated below the alignments. Above the aligned sequences and in the scheme of MecA organisation, the domains N_{1-103} and N_{1-79} are indicated by a red and a violet line, respectively, the linker Li by a black line and the domains C_{121} and C_{100} by an orange and a green line, respectively. Black Stars indicate the conserved residue E_{184} and E_{198} involved in MecA-ClpC interaction in *B. subtilis*. Abbreviations (Genbank accession numbers): MecA_STH, MecA of *S. thermophilus* LMD-9 (YP_819733.1); MecA_MUT, MecA of *S. mutans* UA159 (NP_720709.1); MecA_SPN, MecA of *S. pneumoniae* R6 (NP_358813.1); MecA_BSU, MecA of *B. subtilis* 168 (NP_389034.1).

A

B
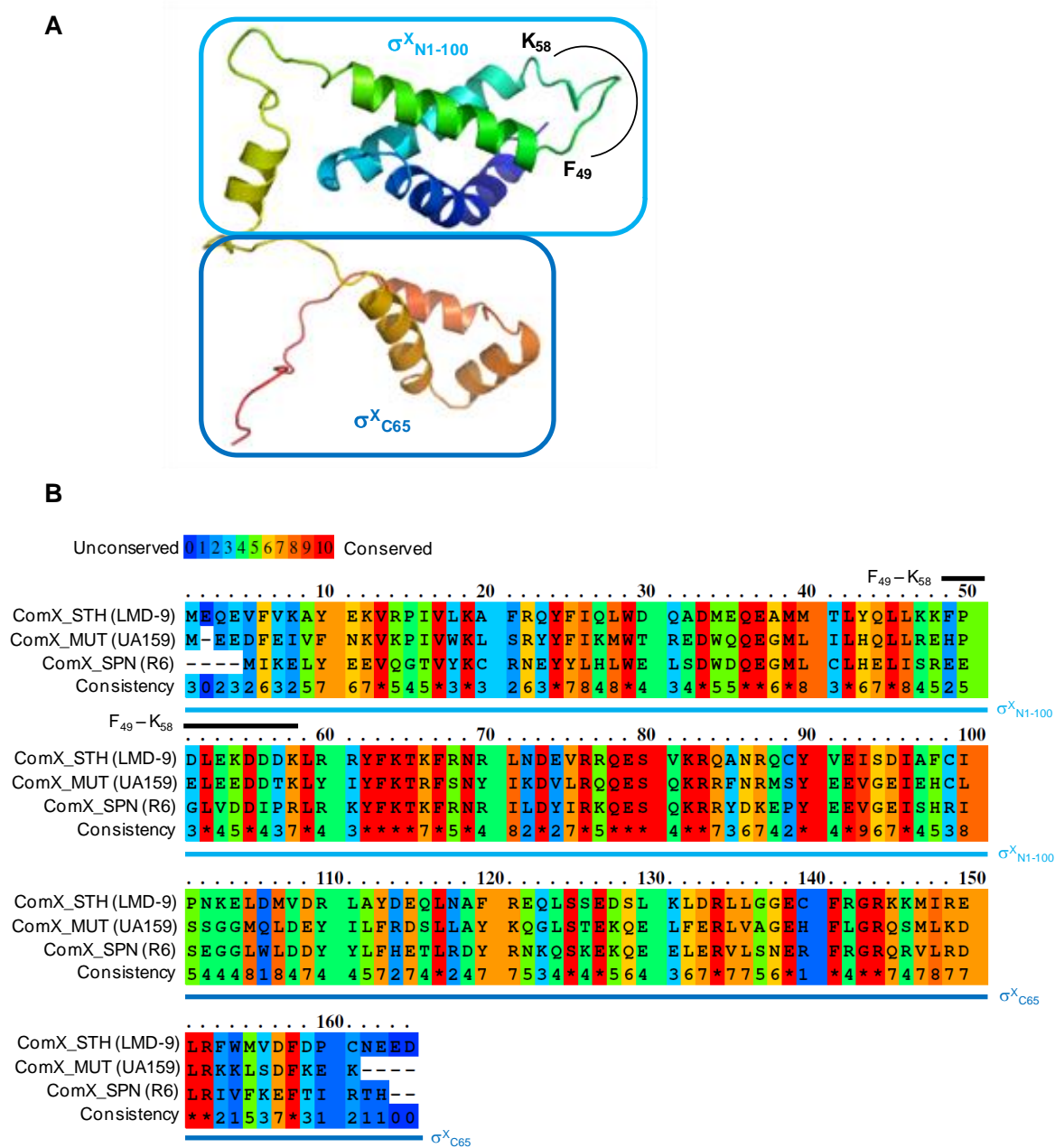
Fig. S2. Predicted structure and protein sequence conservation of σ^X in streptococci (A) Predicted 3D structure of σ^X from *S. thermophilus*. The model was obtained using the LOMETS server (http://zhanglab.ccmb.med.umich.edu/LOMETS/) with σ^E of *E. coli* as template structure (PDB accession number 1OR7). σ^X_{N1-100} (homologous to region 2 of sigma70) and σ^X_{C65} correspond to N (100 aa, surrounded in light blue) and C (65 aa, surrounded in dark blue) domains of σ^X, respectively. The surface-exposed loop F_{49} to K_{58} is indicated by a black semi-circle. (B) Multiple sequence alignment of a selected set of σ^X-like proteins from streptococci. σ^X_{N1-100}, $\sigma XC65$, and the putative surface-exposed loop (F_{49}-K_{58}) are indicated above the aligned sequences as light blue, dark blue, and black lines, respectively. Sequence alignment was performed with the PRALINE package (http://www.ibi.vu.nl/programs/pralinewww/). Conservation is represented by a color code indicated on the top of the alignment where dark blue and red represent the less and most conserved residues, respectively. Similarity scores are indicated below the alignments. Abbreviations (Genbank accession numbers): ComX_STH, σ^X of *S. thermophilus* LMD-9 (YP_819707.1); ComX_MUT, σ^X of *S. mutans* UA159 (NP_722295.1); ComX_SPN, σ^X of *S. pneumoniae* R6 (NP_357607.1).
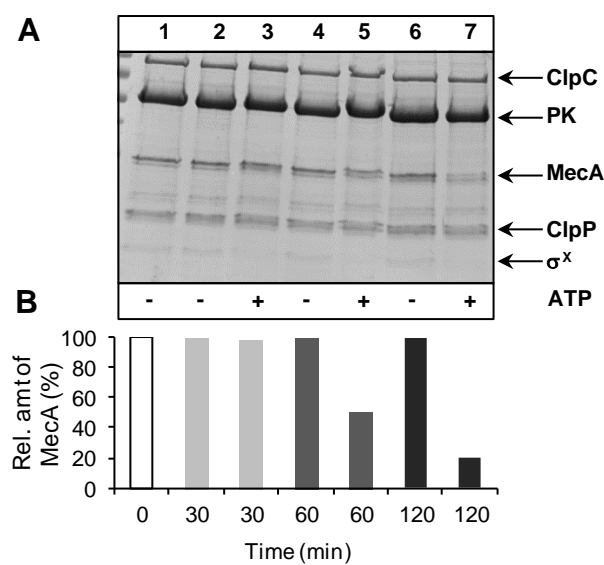
Fig. S3. *In vitro* degradation of MecA and σ^X. (A) SDS-PAGE with Coomassie blue staining of equimolar concentrations (0.6 µM) of 6His-MecA, -ClpC, -ClpE, -ClpP, σ^X-Strep, with or without ATP, and pyruvate kinase/phosphoenolpyruvate (PK/PEP) ATP regeneration system. Samples were taken at 0 (lane 1), 30 (lanes 2 and 3), 60 (lanes 4 and 5), and 120 (lanes 6 and 7) min after adding (+) or not (-) ATP. (B) Quantifications by densitometry of the relative amount of 6His-MecA (%) using control lane 1 as 100%.
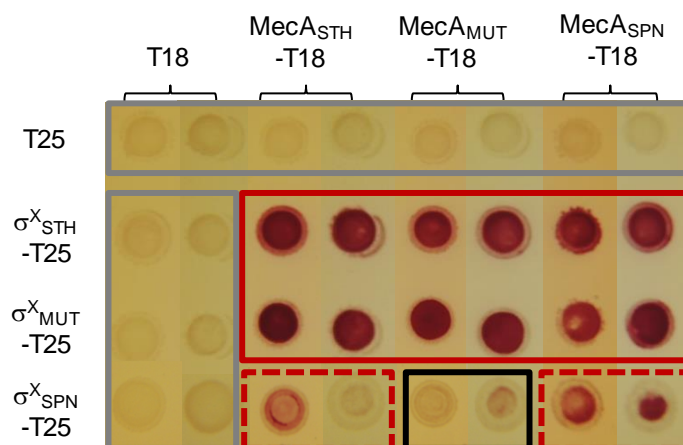
Fig. S4. MecA-σ^X cross-interactions evaluated by B2H on MacConkey indicator plates with cognate pairs of *S. thermophilus* LMD-9, *S. mutans* UA159, and *S. pneumoniae* R6. STH, MUT, or SPN in the index of a protein stands for *S. thermophilus*, *S. mutans*, and *S. pneumoniae*, respectively. Red, dashed red, and black rectangles indicate positive, weak, and negative interactions, respectively. Controls are surrounded by a grey rectangle; T25 and T18 correspond to the empty vectors pKT25 and pUT18, respectively.

REFERENCES

1. **Karimova, G., J. Pidoux, A. Ullmann, and D. Ladant**. 1998. A bacterial two-hybrid system based on a reconstituted signal transduction pathway. Proc. Natl. Acad. Sci. U. S. A **95**:5752-5756.

2. **Sambrook, J., E. Fritsch, and T. Maniatis**. 1989. Molecular cloning: a laboratory manual, second edition. Cold Spring Harbour Laboratory Press, New York, NY, USA.

3. **Boutry, C., A. Wahl, B. Delplace, A. Clippe, L. Fontaine, and P. Hols**. 2012. Adaptor protein MecA is a negative regulator of the expression of late competence genes in *Streptococcus thermophilus*. J. Bacteriol. **194**:1777-1788.

4. **Fontaine, L., C. Boutry, M. H. de Frahan, B. Delplace, C. Fremaux, P. Horvath, P. Boyaval, and P. Hols**. 2010. A novel pheromone quorum-sensing system controls the development of natural competence in *Streptococcus thermophilus* and *Streptococcus salivarius*. J. Bacteriol. **192**:1444-1454.

5. **Karimova, G., A. Ullmann, and D. Ladant**. 2001. Protein-protein interaction between *Bacillus stearothermophilus* tyrosyl-tRNA synthetase subdomains revealed by a bacterial two-hybrid system. J. Mol. Microbiol. Biotechnol. **3**:73-82.

6. **Karimova, G., N. Dautin, and D. Ladant**. 2005. Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. J. Bacteriol. **187**:2233-2243.