

1 Supplementary material

2 Table S1 Bacterial strains and plasmids used in this work

Strain	Description	Reference
COL	MRSA, carrying tetracycline resistance gene (<i>tetK</i>) in pT181 plasmid	[1]
COL h	COL carrying pRIT-sigH	[2]
N315	pre-MRSA	[3]
N315 h	N315 carrying pRIT-sigH	[4]
N315 v	N315 carrying pRIT5H	[4]
N315ex	SCCmec cured derivative of N315	[5]
N315ex-GFP	N315ex carrying pMK3-com-gfp	[2]
N315ex h-GFP	N315ex carrying pRIT-sigH and pMK3-com-gfp	[2]
N315ex ΔsigH-GFP	ΔsigH mutant of N315ex carrying pMK3-com-gfp	This study
N315ex w/oφ	N315ex cured of the φN315 prophage	[2]
N315ex w/oφ h	N315ex w/oφ carrying pRIT-sigH	[2]
N315ex w/oφ ΔcomG h	N315ex w/oφ ΔcomG pRIT-sigH	[2]
N315ex w/oφ ΔcomE h	N315ex w/oφ ΔcomE pRIT-sigH	[2]
<i>E. coli</i> HST04 <i>dam</i> [−] / <i>dcm</i> [−]	<i>E. coli</i> strain lacking the genetic factors <i>dam</i> and <i>dcm</i> that are necessary for	[2]
pHY300	DNA methylation, carrying pHY300PLK (Apm ^R , Tet ^R)	
Plasmids		
pHY300PLK	shuttle vector, ori-pAMa1, Amp ^R (<i>E. coli</i>), Tet ^R (<i>S. aureus</i>)	Takara, Japan
pT181	<i>tetK</i> tetracycline resistance plasmid from COL	[1]
pMADtetsigH	vector for deletion of <i>sigH</i> , Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study

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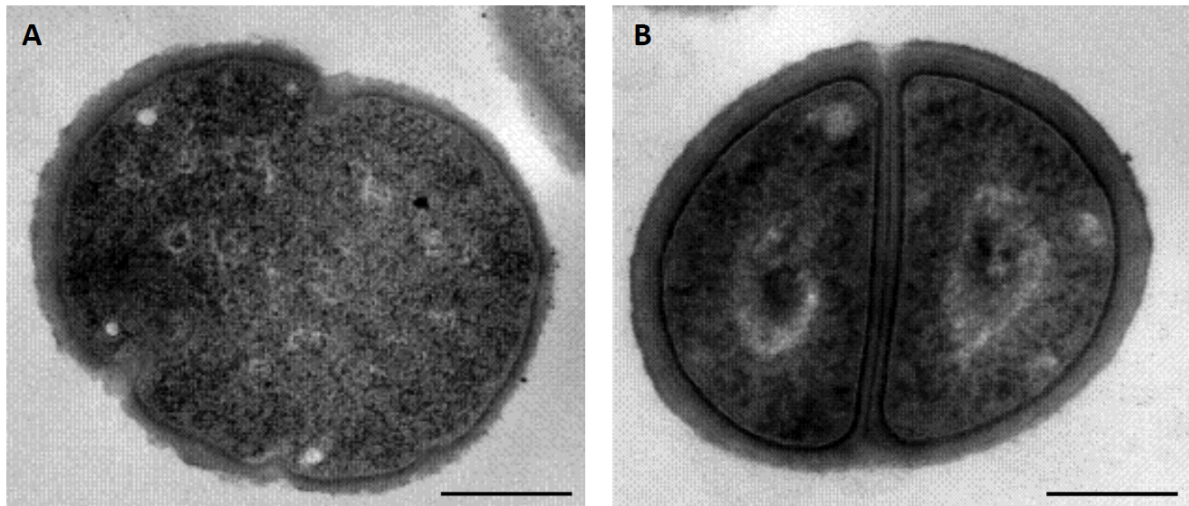


Fig. S1 Transmission electron microscopic images of N315 (A), and COL (B). Cells were cultured for 8 hours in CS2 medium. Scale bar = 0.5 μm

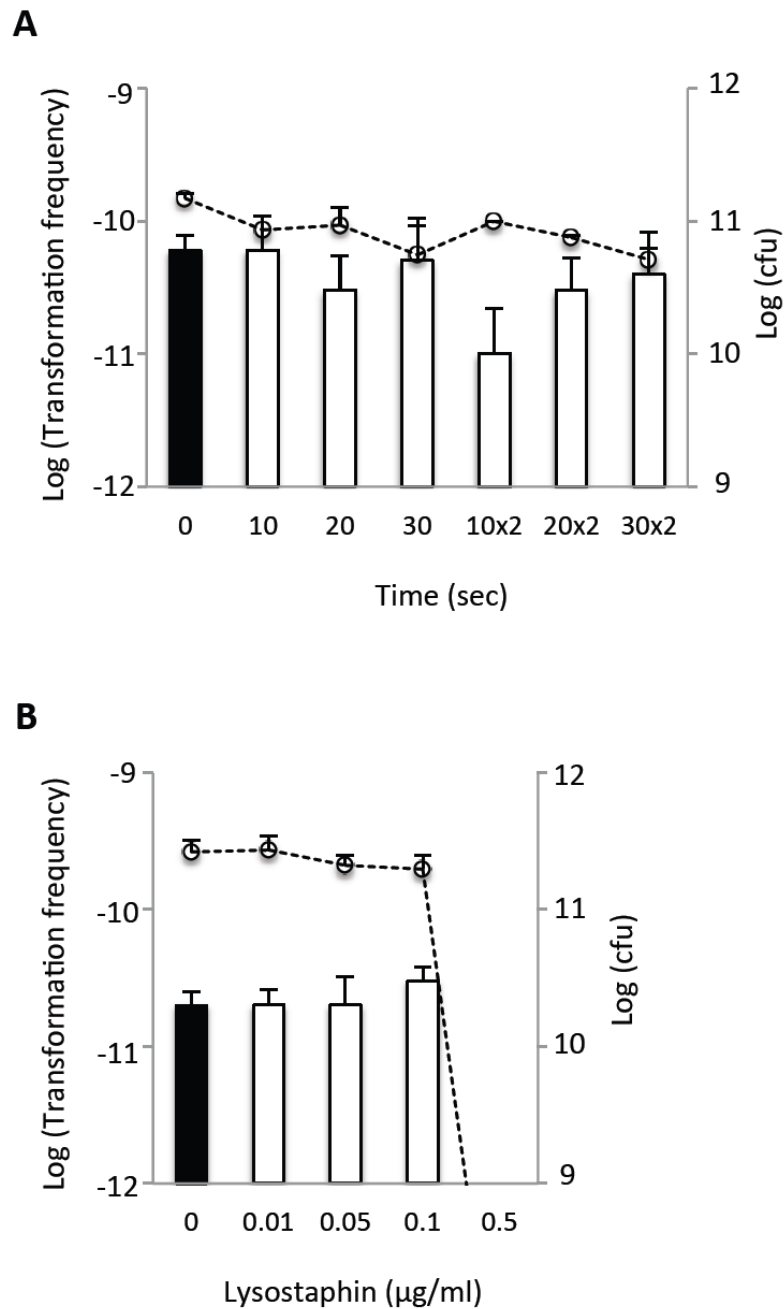


Fig. S2 Transformation frequencies in bead beating or lysostaphin treated cells. (A) Cells were treated by Fastprep device for the indicated periods (0 sec, 10 sec, 20 sec, 30 sec and time double 10x2 sec, 20x2 sec, 30x2 sec) prior to transformation. (B) Cells were incubated with lysostaphin. The values correspond to mean and SD obtained from three independent experiments. Bars: Log_{10} (Transformation frequency); dotted lines: Log_{10} (cfu)

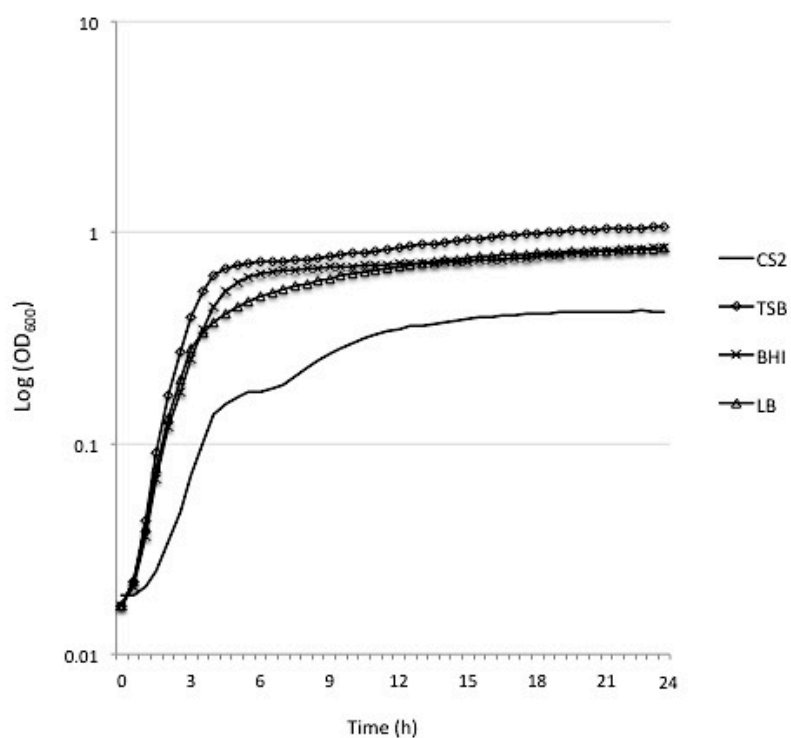


Fig. S3 Growth curves of N315ex in different media. Cells were grown in different culture media on a 96-well plate

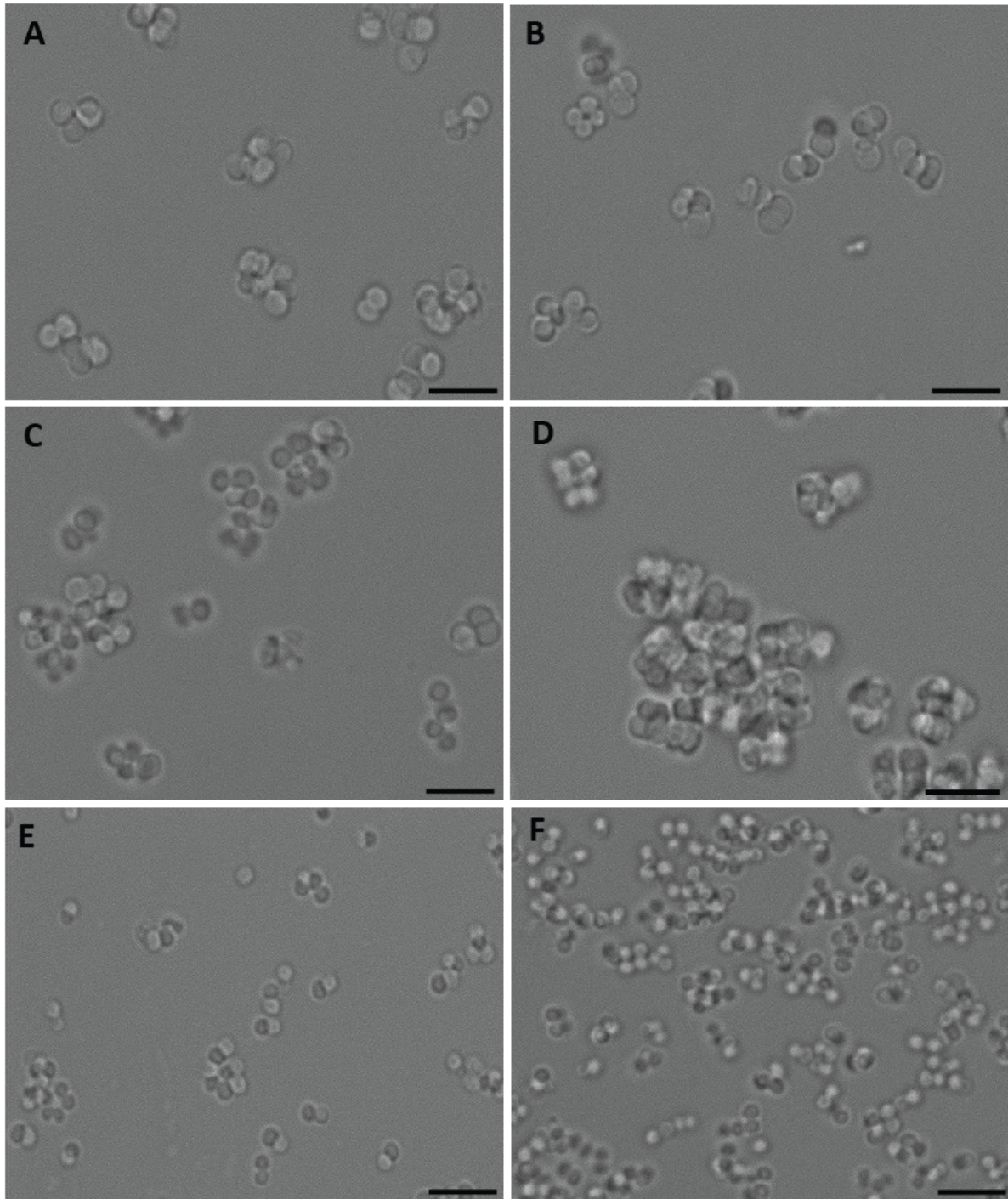


Fig. S4 Phase-contrast microscopic images of N315ex derivative strains cultured 8 hours in CS2 medium (A-D) or TSB (E-F) with (D, F) or without (A-C, E) 0.1% SPS. No morphology difference could be observed between N315ex-GFP (A), N315ex h-GFP (B) and N315ex Δ sigH-GFP (C) grown in CS2. N315ex cells grown in CS2 supplemented with 0.1% SPS (D) show cell aggregation but were not changed in cell size compared to N315ex in normal CS2

(A). No difference could be observed when N315ex was cultured in TSB with 0.1% SPS (F) compared to TSB alone (E). Scale bar = 5 μ m

References for Supplementary material

[1] Dyke KG, Jevons MP, Parker MT. Penicillinase production and intrinsic resistance to penicillins in *Staphylococcus aureus*. Lancet 1966;1:835-8.

[2] Morikawa K, Takemura AJ, Inose Y, Tsai M, Nguyen Thi le T, Ohta T, et al. Expression of a cryptic secondary sigma factor gene unveils natural competence for DNA transformation in *Staphylococcus aureus*. PLoS Pathog. 2012;8:e1003003.

[3] Kuwahara-Arai K, Kondo N, Hori S, Tateda-Suzuki E, Hiramatsu K. Suppression of methicillin resistance in a mecA-containing pre-methicillin-resistant *Staphylococcus aureus* strain is caused by the *mecI*-mediated repression of PBP 2' production. Antimicrob. Agents Chemother. 1996;40:2680-5.

[4] Morikawa K, Inose Y, Okamura H, Maruyama A, Hayashi H, Takeyasu K, et al. A new staphylococcal sigma factor in the conserved gene cassette: functional significance and implication for the evolutionary processes. Genes to cells : devoted to molecular & cellular mechanisms 2003;8:699-712.

[5] Ito, T., Katayama, Y. & Hiramatsu, K. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. 1999; 43:1449-58.