

<Supporting Information>

Antibiotic resistance as a stress response: recovery of high-level oxacillin resistance in methicillin resistant *S. aureus* (MRSA) “auxiliary” mutants – by induction of the stringent stress response

Running title: Stringent stress and oxacillin resistance in MRSA

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Table S1. List of auxiliary mutations reducing the high-level oxacillin resistance in MRSA

Mutation No.	Locus in <i>S. aureus</i> COL	Functional name (<i>gene</i>)	Name of auxiliary mutant ^a	Reference
1	SACOL0181	Hypothetical protein	RUSA162/aux17	(1, 2)
2	SACOL0672	Staphylococcal accessory regulator (<i>sarA</i>)	BB1030 ^b	(3)
3	SACOL0810	Undecaprenyl-phosphate N-acetylglucosaminyl 1-phosphate transferase (<i>Ilm/tarO</i>)	SRM563 ^c	(4)
4	SACOL0828	TPR domain protein	RUSA277/aux18	(1, 2)
5	SACOL0830	Hypothetical protein	RUSA262/aux14	(1, 2)
6	SACOL1023	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase (<i>murE/femF</i>)	RUSA235	(1, 2)
7	SACOL1066	Protein FmtA (<i>fmtA</i>)	COL-TS339	(5)
8	SACOL1231	Protein phosphatase 2C domain protein (<i>ptc1</i>)	SABS1/aux1	(2)
9	SACOL1315	DNA mismatch repair protein MutS (<i>mutS/hexA</i>)	RUSA254/aux9	(1, 2)
10	SACOL1316	DNA mismatch repair protein MutL (<i>mutL/hexB</i>)	RUSA219/aux8	(2)
11	SACOL1329	Glutamine synthetase repressor (<i>glnR/femC</i>)	RUSA208/209	(1, 2)
12	SACOL1377	Transketolase (<i>tkt</i>)	RUSA164/aux6	(1, 2)
13	SACOL1396	Oxacillin resistance-related protein FmtC (<i>fmtC</i>)	COL-TS1	(6)
14	SACOL1398	Transcriptional regulator (<i>msrR</i>)	BB1259 ^b	(7)
15	SACOL1404	Anthranilate synthase component II, glutamine amidotransferase (<i>trpG</i>)	RUSA279/aux5	(1, 2)
16	SACOL1410	Aminoacyltransferase FemA (<i>femA</i>)	BB308/BB403 ^b	(8)
17	SACOL1411	Aminoacyltransferase FemB (<i>femB</i>)	RUSA10	(1)
18	SACOL1424	Phosphate ABC transporter, phosphate-binding protein (<i>pstS</i>)	RUSA305/aux12	(1, 2)
19	SACOL1435	Diaminopimelate decarboxylase (<i>lysA</i>)	RUSA239/aux11	(1, 2)
20	SACOL1444	Hypothetical protein	SABS15/aux3	(2)
21	SACOL1490	Penicillin-binding protein 2 (<i>pbp2</i>)	RUSA130	(1)
22	SACOL1712	Putative ammonia monooxygenase (<i>abrB</i>)	RUSA233/aux10	(1, 2)
23	SACOL1786	Catabolite control protein A (<i>ccpA</i>)	SABS38/aux4	(2)
24	SACOL1801	Putative dipeptidase (<i>pepV</i>)	RUSA188/aux7	(1, 2)
25	SACOL1984	Aldehyde dehydrogenase (<i>aldA2</i>)	RUSA192/aux21	(1, 2)
26	SACOL2026	Accessory gene regulator A (<i>agrA</i>)	BB1165 ^b	(9)
27	SACOL2045	Ketol-acid reductoisomerase (<i>ilvC</i>)	SABS24/aux20	(2)
28	SACOL2054	RNA polymerase sigma-37 factor (<i>rpoF</i>)	RUSA168	(1)
29	SACOL2073	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase (<i>murF</i>)	Mutant F	(10)
30	SACOL2150	Phosphoglucomutase FmtB/Mrp (<i>sasB</i>)	RUSA266	(1)
31	SACOL2151	Phosphoglucosamine mutase (<i>glmM/femD</i>)	RUSA315	(1)
32	SACOL2253	Lipid II:glycine glycytransferase (<i>fmhB/femX</i>)	SR18 ^b	(11)
33	SACOL2630	Putative winged-helix DNA-binding protein	RUSA233/aux16	(1, 2)
34	SACOL2725	ABC transporter, permease protein	RUSA221/aux19	(1, 2)

^a The parental strain of auxiliary mutants is *S. aureus* COL unless indicated.

^b The parental strain is a laboratory strain BB270 (12), a methicillin-resistant derivative of *S. aureus* 8325.

^c Tn918 laboratory mutant of MRSA clinical isolate SRM551 (4).

Table S2. The mupirocin effect is restricted to antibiotic resistance based on the *mecA* determinant

		MIC of oxacillin ($\mu\text{g/ml}$)			
		M100 (no <i>mecA</i>)		COL (<i>mecA</i>)	
Mutated gene	Recipient	none	mupirocin	none	mupirocin
	None		2	2	>256
<i>SACOL0830</i> (RUSA262)		0.25	0.75	1.5	128
<i>murE</i> (RUSA235)		0.75	1.5	1	>256
<i>pbp2</i> (RUSA130)		0.5	0.75	48	>256

MIC was obtained from three experiments independently performed.

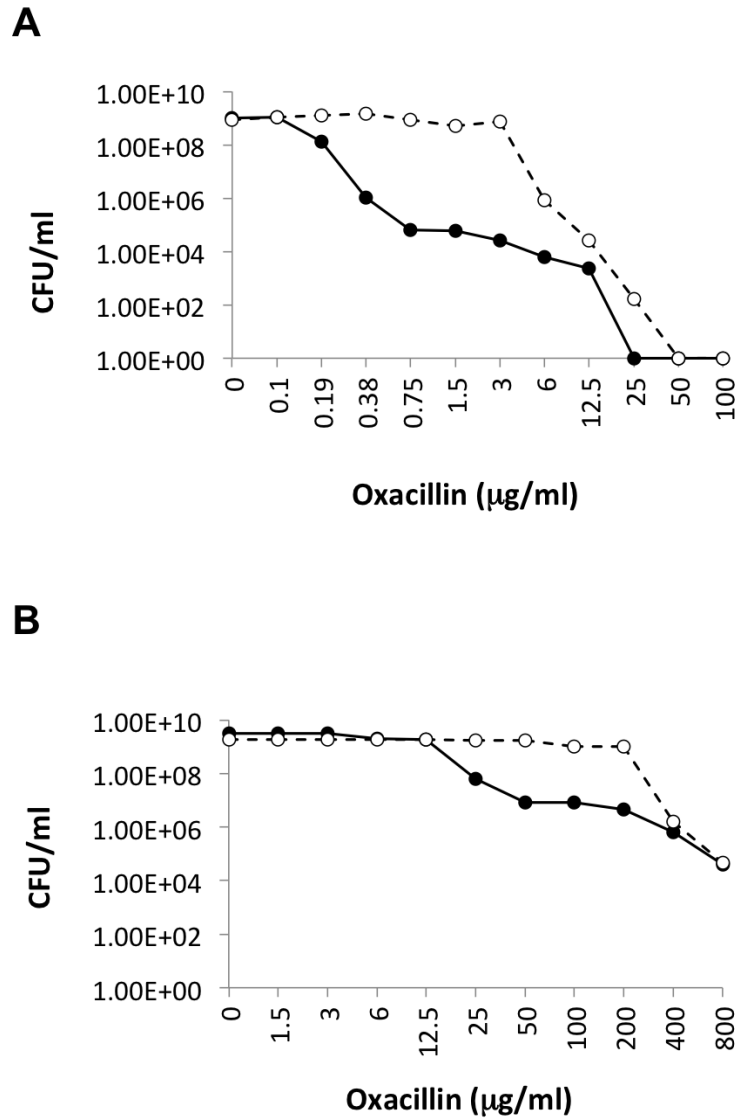


Figure S1. Effect of the stringent stress on MRSA strain LGA251 (**A**) and on *S. sciuri* strain SS37 (**B**), carrying, respectively, *mecC* – in LGA251 (13) – and *mecA1* a putative evolutionary precursor of the *mecA* determinant – in *S. sciuri* SS37 (14, 15). Antibiotic resistance levels were determined by population analysis. Colony-forming units (CFU) were calculated by counting colonies after 48 h of incubation on tryptic soy agar plates with or without 0.03 µg/ml of mupirocin at 37 °C. Closed circles indicate the resistance of strains without induction (●); open circles indicate the resistance of strains induced with mupirocin (○).

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