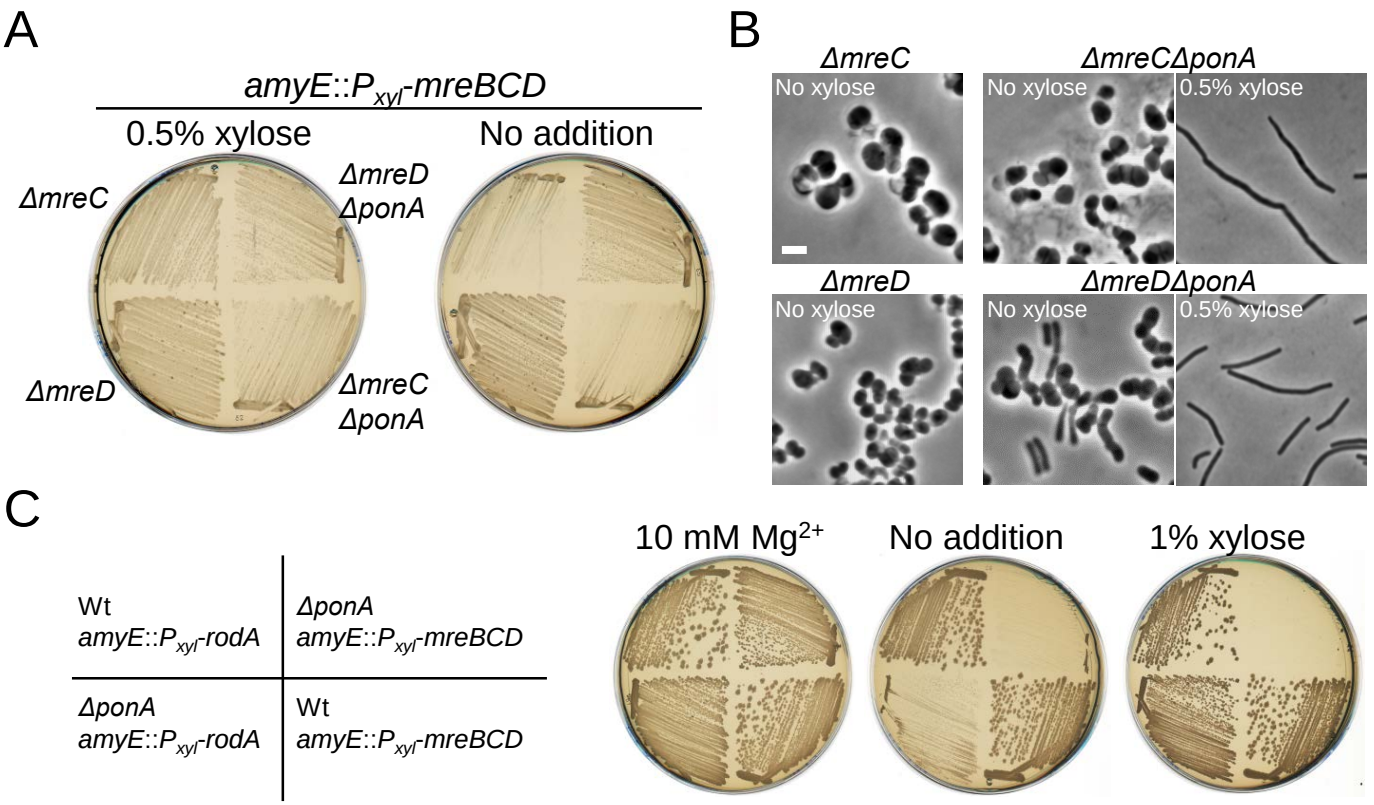
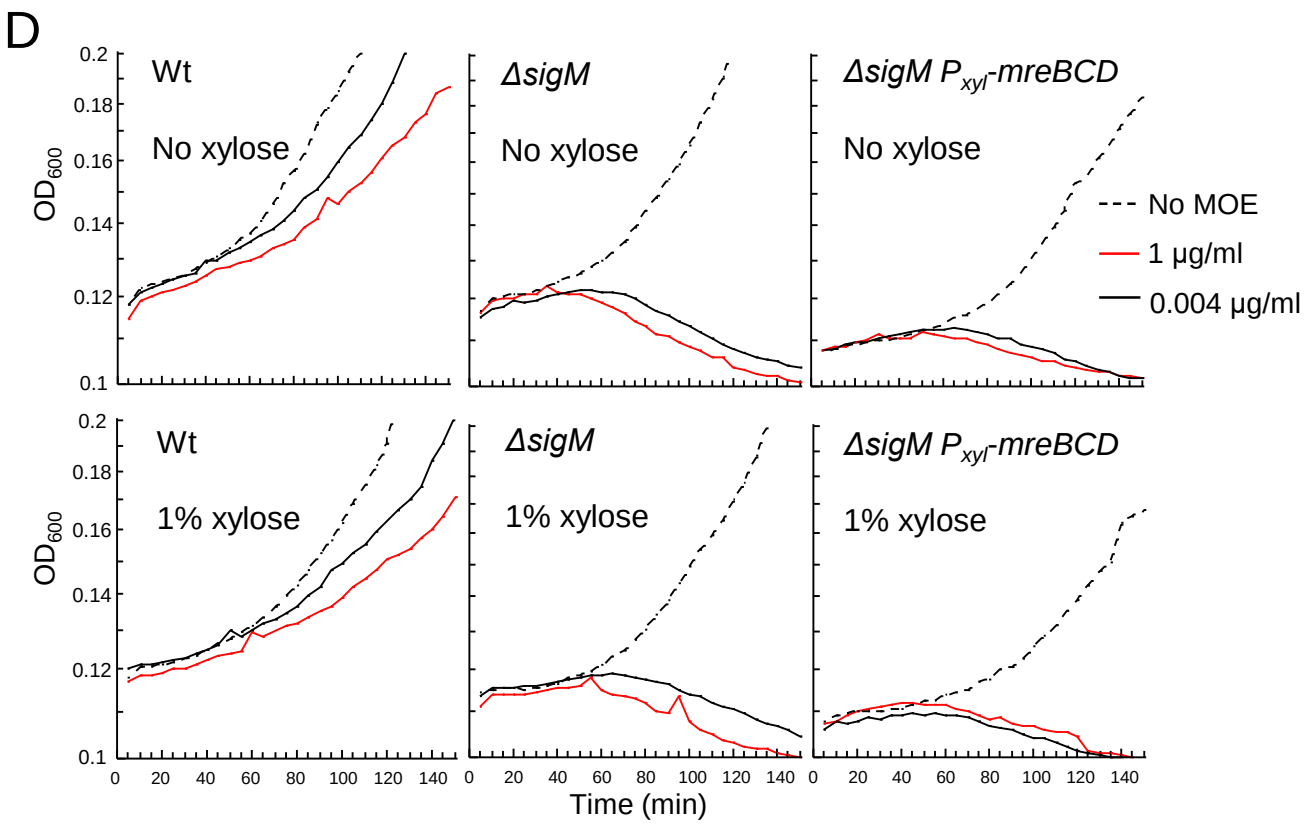
Extended data Figure 1



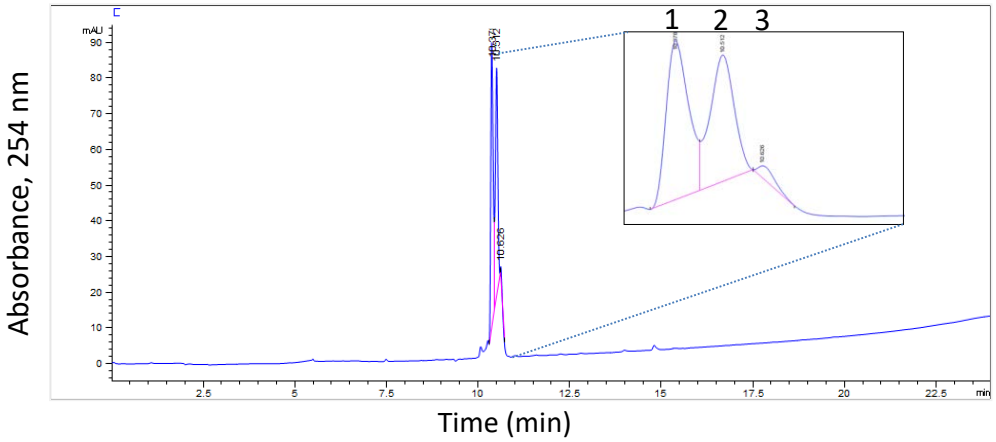


Extended data Figure 2

WT

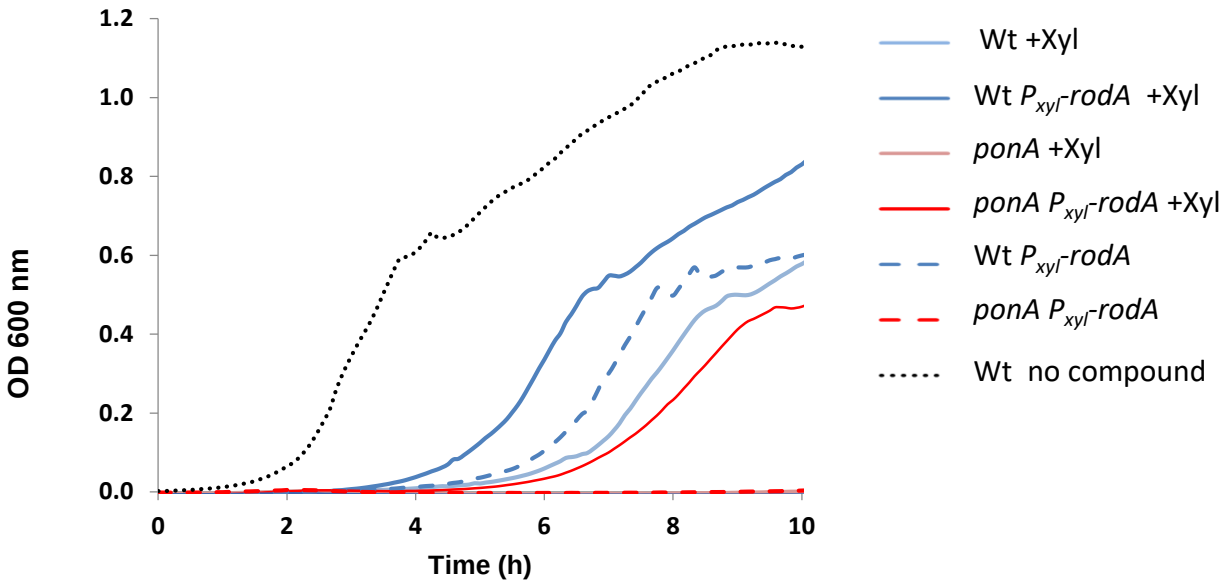
	Antibiotic
1	Ampicillin
2	Bacitracin
3	Cefoxitin
4	Chloramphenicol
5	Doxorubicin
6	Erythromycin
7	Kanamycin
8	Nalidixic acid
9	Nystatin
10	Penicillin
11	Tetracycline
12	Triclosan
13	Spectinomycin
14	Control
15	Citrox

Extended data Figure 3

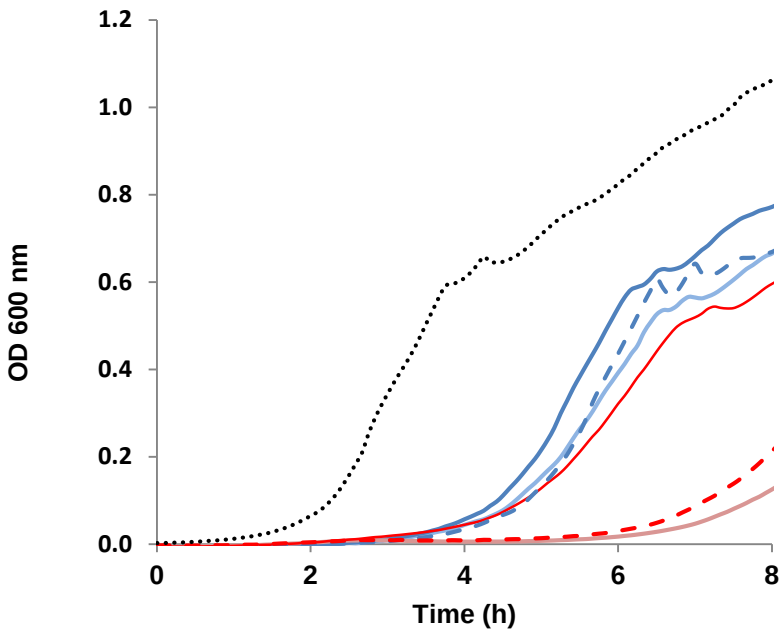


Extended data Figure 4

A



B



Extended data Table 1. Candidates for the missing GTase gene[a].

Gene	BSU code[a]	Operon structure[b]	σ^{M} [c]	MOE[d]	Fi in Δ4 / WT[e]	MOE-growth assays[f]	Known or possible function
divIC	BSU00620		+	+		**Essential**	Unknown (cell division)
tilS	BSU00670	*tilS hprT ftsH coaX yacC*	+	+		**Essential**	tRNA modification
hprT	BSU00680	*tilS hprT ftsH coaX yacC*	+			**Essential**	Nucleotide metabolism
ftsH	BSU00690	*tilS hprT ftsH coaX yacC*	+				Protease
coaX	BSU00700	*tilS hprT ftsH coaX yacC*	+		2.2		Coenzyme A synthesis
yacC	BSU00710	*tilS hprT ftsH coaX yacC*	+		2.8	Tested	Unknown
disA	BSU00880	*disA yacL ispDF*	+	+			Diadenylate cyclase
yacL	BSU00890	*disA yacL ispDF*	+			Tested	Unknown
ispD	BSU00900	*disA yacL ispDF*	+			**Essential**	Lipid II synthesis
ispF	BSU00910	*disA yacL ispDF*	+			**Essential**	Lipid II synthesis
yceC	BSU02890	*yceCDEFGH*	+	+		Tested	Unknown
yceD	BSU02900	*yceCDEFGH*	+	+		Tested	Unknown
yceE	BSU02910	*yceCDEFGH*	+			Tested	Unknown
yceF	BSU02920	*yceCDEFGH*	+			Tested	Unknown
yceG	BSU02930	*yceCDEFGH*	+		1.4	Tested	Unknown
yceH	BSU02940	*yceCDEFGH*	+			Tested	Unknown
ycgQ	BSU03240	*ycgQ ycgR*	+	+	5.7	Tested	Unknown
ycgR	BSU03250	*ycgQ ycgR*	+	+	13.6	Tested	Unknown
amj	BSU04230		+	+	12.2	Tested	Lipid II flipping
ddl	BSU04560	*ddl murF*	+			**Essential**	Lipid II synthesis
murF	BSU04570	*ddl murF*	+			**Essential**	Lipid II synthesis
ydcA	BSU04610			+		Tested	Unknown
ydfM	BSU05470				20.5	Tested	Unknown
yebC	BSU06380		+	+	23.9	Tested	Unknown
ltaSA	BSU07260		+	+	9.7	Tested	Lipoteichoic acid synthesis
yfiC	BSU08220			+		Tested	Unknown
yfiN	BSU08330				21.5	Tested	Unknown

Gene	BSU code[a]	Operon structure[b]	σ^{M} [c]	MOE[d]	Fi in Δ4 / WT[e]	MOE-growth assays[f]	Known or possible function
yhbI	BSU08990				69.9	Tested	Unknown
yhdK	BSU09500	*sigM yhdL yhdK*	+	+	4.7		σ^{M} regulation
yhdL	BSU09510	*sigM yhdL yhdK*	+	+	5.1	**Essential**	σ^{M} regulation
sigM	BSU09520	*sigM yhdL yhdK*	+	+	29.6	Tested	Transcriptional regulation
yjbC	BSU11490	*yjbC spx*	+			Tested	Unknown
spx	BSU11500	*yjbC spx*	+				Transcriptional regulation
abh	BSU14480		+	+	1.7	Tested	Transcriptional regulation
ylaA	BSU14710			+	1.9	Tested	Unknown
ylzH	BSU15069				13.1	Tested	Unknown
murG	BSU15220	*murGB divIB ylxWX sbp*	+	+	14.3	**Essential**	Lipid II synthesis
murB	BSU15230	*murGB divIB ylxWX sbp*	+	+	12.1	**Essential**	Lipid II synthesis
divIB	BSU15240	*murGB divIB ylxWX sbp*	+	+	5.9	**Essential**	Unknown (cell division)
ylxW	BSU15250	*murGB divIB ylxWX sbp*	+	+	4.5	Tested	Unknown
ylxX	BSU15260	*murGB divIB ylxWX sbp*	+	+	3.4	Tested	Unknown
sbp	BSU15270	*murGB divIB ylxWX sbp*	+	+		Tested	Unknown
pbpX	BSU16950		+	+	2.3		PG turnover
yngC	BSU18190		+		2.9	Tested	Unknown
metA	BSU21910	*metA ugtP*	+		-1.8		Methionine synthesis
ugtP	BSU21920	*metA ugtP*	+		1.5		Glucolipid synthesis
recU	BSU22310	*recU ponA*	+	+	5.9		DNA recombination
ponA	BSU22320	*recU ponA*	+	+	-186.5		PG synthesis
ypbG	BSU22980		+	+	5.0	Tested	Unknown
ypuD	BSU23300		+	+	5.1	Tested	Unknown
ypuA	BSU23370		+		11.0	Tested	Unknown
yqjL	BSU23830		+	+	4.0	Tested	Unknown
yqiG	BSU24210			+		Tested	Unknown
yrhL	BSU27140			+	1.5	Tested	Unknown
yrhJ	BSU27160	*yrhH fatR yrhJ*	+	+	2.6	Tested	Unknown
fatR	BSU27170	*yrhH fatR yrhJ*	+	+	1.5	Tested	Transcriptional regulation

Gene	BSU code[a]	Operon structure[b]	σ^{M} [c]	MOE[d]	Fi in Δ4 / WT[e]	MOE-growth assays[f]	Known or possible function
yrhH	BSU27180	*yrhH fatR yrhJ*	+	+	15.9	Tested	Unknown
secDF	BSU27650		+				Protein secretion
yrzF	BSU27785			+	2.1	Tested	Unknown
minD	BSU27990	*maf radC mreBCD minCD*	+		3.5	Tested	Cell division regulation
minC	BSU28000	*maf radC mreBCD minCD*	+	+	3.7	Tested	Cell division regulation
mreD	BSU28010	*maf radC mreBCD minCD*	+	+	5.7	**Essential**	Unknown (cell shape)
mreC	BSU28020	*maf radC mreBCD minCD*	+	+	6.2	**Essential**	Unknown (cell shape)
mreB	BSU28030	*maf radC mreBCD minCD*	+	+	5.0		Cytoskeleton
radC	BSU28040	*maf radC mreBCD minCD*	+		7.6		DNA repair
maf	BSU28050	*maf radC mreBCD minCD*	+	+	6.8		Unknown
ytpB	BSU30500	*ytpA ytpB*	+				Unknown
ytpA	BSU30510	*ytpA ytpB*	+	+		Tested	Unknown
yvrD	BSU33190			+		Tested	Unknown
tagT	BSU35840		+	+	2.3	Tested	Teichoic acid synthesis
rapD	BSU36380		+		-1.8		Competence regulation
bcrC	BSU36530		+	+	5.7	Tested	Lipid II recycling
ywnJ	BSU36540		+	+	1.3	Tested	Unknown
ywzG	BSU38018				12.7	Tested	Unknown
rodA	BSU38120		+	+	4.7	**Essential**	Unknown (cell shape)
ywbO	BSU38250	*efeB ywbO*	+		-1.4	Tested	Unknown
efeB	BSU38260	*efeB ywbO*	+				Iron uptake
ywbG	BSU38330				16.5	Tested	Unknown
ywaC	BSU38480		+	+	5.3	Tested	(p)ppGpp synthase
dltA	BSU38500	*dltABCDE*	+		3.6	Tested	Teichoic acid synthesis
dltB	BSU38510	*dltABCDE*	+		6.2	Tested	Teichoic acid synthesis
dltC	BSU38520	*dltABCDE*	+		7.7		Teichoic acid synthesis
dltD	BSU38530	*dltABCDE*	+		7.7		Teichoic acid synthesis
dltE	BSU38540	*dltABCDE*	+	+	7.3		Teichoic acid synthesis
yxeF	BSU39570				24.4	Tested	Unknown

a Genes and BSU code organized by increasing BSU number.

b A few σ^M-dependent genes belong to same operon [10 26,27], as indicated.

c Known σ^M-dependent genes are labelled "+" [10 26].

d Genes reported to belong to the MOE stimulon are labelled "+"[10].

e Fold increase (Fi) in transcription (RNA-Seq data) of genes in strain *Δ4* (AG157) relative to the wild type (168CA), in mid-exponential cells grown in nutrient broth at 37°C. "-" indicates down regulation.

f Around fifty gene deletion strains were tested by MOE-growth assays (growth in LB was monitored for 16h in the presence of 0, 1.25, 2.5, 5, 10 and 20 μg ml^{-1} of MOE). Essential genes were not tested, except as indicated in the Results.

Extended data Table 2. Strains plasmids and oligos.

Bacterial strains used in this study

Strains	Relevant genotype	References
E. coli		
DH5α	F- ϕ80*lacZΔM15* Δ(*lacZYAargF*)U196 *recA1 endA1 hsdR17* (r_{K-}, m_{K+}) *phoA supE44* λ- *thi1*	Lab. stock
TG1	K-12 *supE thi-1 Δ*(*lac-proAB*) *Δ*(*mcrB-hsdSM*)*5,* ($r_K^-m_K^-$)	Lab. stock
B. subtilis		
168CA	*trpC2*	Lab. stock
DPVB45	*ΔpbpG*::*kan*	[11]
RE101	168CA *ΔponA*	This work
AG157	168CA *ΔponA ΔpbpD ΔpbpF ΔpbpG*::*kan*	This work
AG201	168CA *ΔpbpG*::*erm*	This work
AG221	168CA *ΔponA ΔpbpD ΔpbpF ΔpbpG*::*erm* pLOSS-P_{spac}-*ponA*	This work
AG494	168CA *ΔsigM*::*kan*	[28]
AG831	AG221 *ΔsigM*::*kan*	This work
YK1342	168CA *Ωneo3427 ΔmreD*	[16]
YK1343	168CA *ΔrodA*::*kan*	[16]
YK1347	168CA *Ωneo3427 ΔmreC kan*	[16]
YK1334	168CA *ΔponA*::*spc*	[24]
YK2235	AG221 Ω*rodA*::*kan*-P_{spac}-*rodA*	This work
YK2239	168CA *ΔponA ΔpbpD ΔpbpF ΔpbpG*::*erm*	This work
YK2242	YK2239 Ω*rodA*::*kan*-P_{spac}-*rodA*	This work
YK2245	168CA Ω*rodA*::*kan*-P_{spac}-*rodA*	This work
YK2246	168CA *ΔponA*::*spc* Ω*rodA*::*kan*-P_{spac}-*rodA*	This work
YK2249	168CA *amyE*::P_{xyl}-*rodA cat*	This work
YK2250	168CA *amyE*::P_{xyl}-*ftsW cat*	This work
YK2251	168CA *ΔponA*::*spc amyE*::P_{xyl}-*rodA cat*	This work
YK2256	AG221 *amyE*::P_{xyl}-*rodA cat*	This work
YK2257	AG221 *amyE*::P_{xyl}-*ftsW cat*	This work
YK2259	AG221 *amyE*::P_{xyl}-*rodA cat ΔsigM*::*kan*	This work
YK2268	168CA *amyE*::P_{xyl}-*rodA cat ΔsigM*::*kan*	This work
YK2270	168CA *amyE*::P_{xyl}-*mreBCD spc ΔmreC*::*kan*	[15]
YK2271	168CA *amyE*::P_{xyl}-*mreBCD spc ΔmreD*::*kan*	[15]
YK2272	168CA *amyE*::P_{xyl}-*mreBCD spc ΔmreC*::*kan ΔponA*	This work
YK2273	168CA *amyE*::P_{xyl}-*mreBCD spc ΔmreD*::*kan ΔponA*	This work
YK2274	168CA *amyE*::P_{xyl}-*mreBCD*	[15]
YK2275	168CA *amyE*::P_{xyl}-*mreBCD spc ΔponA*	This work
YK2276	168CA *ΔsigM*::*kan amyE*::P_{xyl}-*mreBCD spc*	This work
B. subtilis **strains used for MOE sensitivity test**		
AG832	168CA *ΔylxW*::*erm*	Koo BM *et al.*, in preparation
AG833	168CA *ΔylxX*::*erm*	Koo BM *et al.*, in preparation
AG834	168CA *Δsbp*::*erm*	Koo BM *et al.*, in preparation
AG835	168CA *ΔyqjL*::*erm*	Koo BM *et al.*, in preparation
AG836	168CA *ΔytpA*::*erm*	Koo BM *et al.*, in preparation
AG837	168CA *ΔyceC*::*erm*	Koo BM *et al.*, in preparation
AG838	168CA *ΔyceD*::*erm*	Koo BM *et al.*, in preparation
AG839	168CA *ΔyceE*::*erm*	Koo BM *et al.*, in preparation
AG840	168CA *ΔypbG*::*erm*	Koo BM *et al.*, in preparation
AG851	168CA *ΔypuD*::*erm*	Koo BM *et al.*, in preparation
AG852	168CA *ΔyqiG*::*erm*	Koo BM *et al.*, in preparation
AG853	168CA *ΔyxeF*::*erm*	Koo BM *et al.*, in preparation
AG856	168CA *ΔyceH*::*erm*	Koo BM *et al.*, in preparation
AG857	168CA *ΔyceF*::*erm*	Koo BM *et al.*, in preparation
AG858	168CA *ΔyfiC*::*erm*	Koo BM *et al.*, in preparation
AG859	168CA *ΔylzH*::*erm*	Koo BM *et al.*, in preparation

AG860	168CA *ΔyrzF*::*erm*	Koo BM *et al.*, in preparation
AG861	168CA *ΔywbG*::*erm*	Koo BM *et al.*, in preparation
AG862	168CA *ΔywzG*::*erm*	Koo BM *et al.*, in preparation
AG863	168CA *ΔydfM*::*erm*	Koo BM *et al.*, in preparation
AG864	168CA *ΔyrhH*::*erm*	Koo BM *et al.*, in preparation
AG865	168CA *ΔyfiN*::*erm*	Koo BM *et al.*, in preparation
AG881	168CA *ΔycgQ*::*erm*	Koo BM *et al.*, in preparation
AG882	168CA *ΔycgR*::*erm*	Koo BM *et al.*, in preparation
AG883	168CA *ΔyjbC*::*erm*	Koo BM *et al.*, in preparation
AG884	168CA *ΔylaA*::*erm*	Koo BM *et al.*, in preparation
AG885	168CA *Δamj*::*erm*	Koo BM *et al.*, in preparation
AG886	168CA *ΔyrhL*::*erm*	Koo BM *et al.*, in preparation
AG887	168CA *ΔytrB*::*erm*	Koo BM *et al.*, in preparation
AG888	168CA *ΔywbO*::*erm*	Koo BM *et al.*, in preparation
AG1085	168CA *ΔyceG*::*erm*	Koo BM *et al.*, in preparation
AG1086	168CA *ΔywaC*::*erm*	Koo BM *et al.*, in preparation
AG1087	168CA *ΔyacL*::*erm*	Koo BM *et al.*, in preparation
AG1088	168CA *ΔyebC*::*erm*	Koo BM *et al.*, in preparation
AG1089	168CA *Δabh*::*erm*	Koo BM *et al.*, in preparation
AG1090	168CA *ΔyacC*::*erm*	Koo BM *et al.*, in preparation
AG1091	168CA *Δypu*A::*erm*	Koo BM *et al.*, in preparation
AG1092	168CA *ΔyngC*::*erm*	Koo BM *et al.*, in preparation
AG1093	168CA *ΔydcA*::*erm*	Koo BM *et al.*, in preparation
AG1094	168CA *ΔbcrC*::*erm*	Koo BM *et al.*, in preparation
AG1095	168CA *ΔyrhJ*::*erm*	Koo BM *et al.*, in preparation
AG1096	168CA *ΔfatR*::*erm*	Koo BM *et al.*, in preparation
AG1097	168CA *ΔywnJ*::*erm*	Koo BM *et al.*, in preparation
AG1098	168CA *ΔyvrD*::*erm*	Koo BM *et al.*, in preparation
AG1100	168CA *ΔyhbI*::*erm*	Koo BM *et al.*, in preparation
AG1161	168CA *ΔminCD*::*kan*	[29]
AG1174	168CA *minD*::*erm*	[30]
AG1245	168CA *dltAB*::*cat*	[31]
RE203	168CA *ΔtagT*::*spc*	[16]
AG612	168CA *ΔltaSA*::*erm*	[32]

cat, chloramphenicol; *tet*, tetracyclin; *erm*, erythromycin; *spc*, specctinomycin; *kan*, kanamycin; *bla*, β-lactamase; represent resistant genes, respectively.

Plasmids used in this study

Plasmids	**Relevant features**	**References**
pMUTIN4	used as matrix to amplify *erm*	[33]
pG$^+$host9	thermosensitive replication, *erm*	[23]
pG$^+$host10	pG$^+$host9, *bla*	This study
pG$^+$host9::*ΔponA*	carry flanking DNA regions of *ponA*	This study
pG$^+$host10::*ΔpbpD*	carry flanking DNA regions of *pbpD*	This study
pG$^+$host10::*ΔpbpF*	carry flanking DNA regions of *pbpF*	This study
pLOSS*	*bla spc* P_{spac}-*mcs* P_{divIVA}-*lacZ lacI* rep_{pLS20}(*GA→CC*)	[24]
pLOSS-P_{spac}-*ponA*	*bla spc* P_{spac}-*ponA* P_{divIVA}-*lacZ lacI* rep_{pLS20}(*GA→CC*)	This study
pSG441	*bla aph-A3 lacI* P_{spac}	[34]
pRD159	*bla aph-A3 lacI* P_{spac}-*rodA'*	This work
pJPR1	*bla amyE3' cat* P_{xyl} *amyE5'*	Lab stock
pRD163	*bla amyE3' cat* P_{xyl}*–rodA amyE5'*	This work
pRD164	*bla amyE3' cat* P_{xyl}*–ftsW amyE5'*	This work

erm, erythromycin resistance*, bla,* ampicillin resistance; *spc*, spectinomycin resistance; *mcs*, multiple cloning site; *lacZ*, β-galactosidase ; *lacI*, lactose repressor.

Primers used in this study

Primers	**Sequence**	
RE05	GACATCGATGACGAAAGGGCCTCGTGATACGC	To insert *bla* into pGhost9
RE06	CTTACTCGAGCCACCGCTGGTAGCGGTGG	To insert *bla* into pGhost9

RE01	GACGCGGCCGCCGAAGATGACTTAAACGAAACG	*ponA* pGhost9 left flank For primer (NotI)
RE02	CTTAGAATTCCGTTAATCAACCTTTCGCACC	*ponA* pGhost9 left flank Rev primer (EcoRI)
RE03	GACGAATTCCCGTCACCTTTGGGGTGATGGC	*ponA* pGhost9 right flank For primer (EcoRI)
RE04	CTTAGGTACCCGCAAGCAAAAGAAGTGTTGGC	*ponA* pGhost9 right flank Rev primer (KpnI)
RE07	GACGCGGCCGCGGCATTTTCAGCAACGGG	*pbpD* pGhost9 left flank For primer (NotI)
RE08	CTTAGAATTCATTCTAGCCATATCGTA	*pbpD* pGhost9 left flank Rev primer (EcoRI)
RE09	GACGAATTCTAAAGGATCGGAGGGGATATGG	*pbpD* pGhost9 right flank For primer (EcoRI)
RE10	CTTAAAGCTTCTAGATTCTCAGGTTTCCGCCG	*pbpD* pGhost10 right flank Rev primer (HindIII)
RE11	GACGCGGCCGCGCCAGTCTTGAAAGGCGCTTTCCG	*pbpF* pGhost9 left flank For primer (NotI)
RE12	CTTAGAATTCTTCTAAAGGTAGTATGGG	*pbpF* pGhost9 left flank Rev primer (EcoRI)
RE13	GACGAATTCGCGATTTTTTGAACTTTGAGC	*pbpF* pGhost9 right flank For primer (EcoRI)
RE14	CTTAAAGCTTCTCAAGCACGTACGGCACGTCC	*pbpF* pGhost10 right flank Rev primer (HindIII)
AG21B	GGTAACGCCAGGGTTTTC	To check insert in pGhost9 by sequencing
AG22	GTGCCACCTGACGTCTAA	To check insert in pGhost9/10 by sequencing
AG23	CACGTTACTAAAGGGAATG	To check insert in pGhost10 by sequencing
AG11	GACGCGGCCGCGAAAGGTTGAGATGTTATGTC	To amplify *ponA* locus
AG12	GGCACTAGTTAAAACGGGAGGTTTTTTATTGTG	To amplify *ponA* locus
AG18	GTTGACTTTATCTACAAGGT	To check insert cloned in pLOSS*
AG19	GGTACCAGTAGTTCACCAC	To check insert cloned in pLOSS*
AG124	CCATCATCTGGTGCGAAAGG	To check *B. subtilis ΔponA*
AG125	CCGCAAAGCCGATTAATTGG	To check *B. subtilis ΔponA*
AG126	TCTATTGGCGAGTGCTTC	To check *B. subtilis ΔpbpF*
AG127	AGCATCGACTCCGTATTG	To check *B. subtilis ΔpbpF*
AG128	TTATTCGGAATGGCGATGGG	To check *B. subtilis ΔpbpD*
AG129	CCTTAATGGCTGCAGTTGAC	To check *B. subtilis ΔpbpD*
AG203	GTACGTTCTAGAGTGTTCGTGCTGACTTGC	To amplify erythromycin cassette
AG204	GGGGGATCCTTGGAAGCTGTCAGTAG	To amplify erythromycin cassette
AG200-for	GACAGGCCATGAAATTGGAG	To amplify upstream *pbpG*
AG114-rev	GCTCTAGACATTGCATCCACAACGGGTTCC	To amplify upstream *pbpG*
AG201	CGGGATCCGGACAGAACCCGCAAACGTG	To amplify downstream *pbpG*
AG202	TGGGAGCTGCACCATAAAGG	To amplify downstream *pbpG*
AG130	AGAACGTGCTCCGGATTTGG	To check *B. subtilis ΔpbpG*
AG131	CGGCGACTGGGAGATGAAAG	To check *B. subtilis ΔpbpG*
AG338	TTCCATTGTGCCACTCCTTC	To check *B. subtilis ΔsigM*
AG339	GTTCGCTGTCAATCAGTTCC	To check *B. subtilis ΔsigM*
AG328	AAGTCTAGATAATGGAAGTTAGAAGCAGGGAAGAGGATG	To amplify *ftsW*
AG329	ATTAGCGGCCGCGGAATATCCTTCCCCTGTACAC	To amplify *ftsW*
AG330	ATGTCTAGATGAAAATGTAAGGCGGGATAGAATGAG	To amplify *rodA*
AG331	GATGCGGCCGCAGCCTCTGTAAAGGCTGTCTGC	To amplify *rodA*