Supplementary Tables and Plasmid Construction

Table S1: Strains used in this study

Strain	Genotype	Reference
BJH001	PY79 (wild-type)	(1)
BQA003	Δ*yjbM*::*erm*	This work
BQA006	Δ*ywaC*::*erm*	This work
BQA009	Δ*relA*::*spec*	This work
BQA010	Δ*relA*::*erm*	This work
BQA022	Δ*sigD*::*tet*	(2)
BQA046	*amyE*::P_{hag}*-lacZ (cat)*	This work
BQA047	*amyE*::P_{lytA}*-lacZ (cat)*	This work
BQA050	*amyE*::P_{lytA}*-lacZ (cat),* Δ*relA*::*spec*	This work
BQA051	*amyE*::P_{lytA}*-lacZ (cat),* Δ*relA*::*spec*	This work
BQA057	$hag^{HagT209C}$	This work
BQA059	Δ*relA*::*spec* / Δ*sigD*::*tet*	This work
BQA062	$hag^{HagT209C}$, Δ*relA*::*spec*	This work
BQA067	*amyE*::P_{relA}*-relA (spec)*	This work
BQA068	*amyE*::P_{relA}*-relA (spec),* Δ*relA*::*erm*	This work
BQA071	*amyE*::P_{hag}*-lacZ (cat),* Δ*sigD*::*tetR*	This work
BQA072	*amyE*::P_{lytA}*-lacZ (cat),* Δ*sigD*::*tetR*	This work
BQA073	*amyE*::P_{hag}*-lacZ (cat), sacA*::P_{relA}*-relA (spec),* Δ*relA*::*erm*	This work
BQA074	*amyE*::P_{lytA}*-lacZ (cat), sacA*::P_{relA}*-relA (spec),* Δ*relA*::*erm*	This work
BQA075	*sacA*::P_{relA}*-relA (spec),* Δ*relA*::*erm*	This work
BQA076	Δ*hag::erm*	*Bacillus* Genetic Stock Center
BQA080	$hag^{HagT209C}$*, amyE*::P_{relA}*-relA (spec),* Δ*relA*::*spec*	This work
BQA081	Δ*relA*::*spec,* Δ*yjbM*::*erm*	This work
BQA082	Δ*relA*::*spec,* Δ*ywaC*::*erm*	This work
BQA083	*amyE*::P_{hy}*-sigD (kan),* Δ*sigD*::*tet,* Δ*relA*::*erm*	This work
BQA084	*amyE*::P_{hag}*-lacZ (cat),* Δ*sigD*::*tetR,* Δ*relA*::*spec*	This work
BQA085	*amyE*::P_{lytA}*-lacZ (cat),* Δ*sigD*::*tetR,* Δ*relA*::*spec*	This work
BQA086	*amyE*::P_{hag}*-lacZ (cat),* Δ*relA*::*spec,* Δ*yjbM*::*erm*	This work
BQA087	*amyE*::P_{lytA}*-lacZ (cat),* Δ*relA*::*spec,* Δ*yjbM*::*erm*	This work
BQA088	*amyE*::P_{hag}*-lacZ (cat),* Δ*relA*::*spec,* Δ*ywaC*::*erm*	This work
BQA089	*amyE*::P_{lytA}*-lacZ (cat),* Δ*relA*::*spec,* Δ*ywaC*::*erm*	This work
DS874	*amyE*::P_{hy}*-sigD (kan)*	Daniel B. Kearns
EUB004	*trpC2* Δ*relA::erm, aprE::Pspac-*$relA_{D264G}$ *(spec)*	(3)

Table S2: Plasmids used in this study

Plasmid	Description	Reference
pDR111	*amyE::*P*hyperspank (spec) (amp)*	David Z. Rudner
pMiniMAD2	ori^{BsTs} *(amp) (erm)*	(4)
pDG1661	*amyE::lacZ (cat)*	*Bacillus* Genetic Stock Center
pJW053	Δ*yjbM::spec*	This work
pJW054	Δ*relA::spec*	This work
pJW055	Δ*yjbM::erm*	This work
pJW058	Δ*relA::erm*	This work
pJW063	Δ*ywaC::spec*	This work
pJW064	Δ*ywaC::erm*	This work
pKM079	*B. subtilis chromosomal integration vector (spec)*	David Z. Rudner
pKM082	*B. subtilis chromosomal integration vector (erm)*	David Z. Rudner

pQA014	*amyE::*P_{lytA}*-lacZ (cat)*	This work
pQA015	*amyE::*P_{hag}*-lacZ (cm)*	This work
pQA017	*hag*^{HagT209C}	This work
pQA020	Δ*amyE::*P_{relA}*-relA (spec)*	This work

Table S3: Oligonucleotides used in this study

Primer	Sequence (5' to 3')
oJW052	GGCCGGCCGTGCTCTTCCTTTCCGCCCTGT
oJW053	CATGTCGACTCCCCCAATTCCGAACCAGTT
oJW054	GCAGGATCCGGTAAAGGGGAAGAAGAGCATG
oJW055	GATGAATTCTCCGCCCAGCGCCTTATT
oJW056	GAACGGCCGGGCTTTATTATCGGCTGTCCC
oJW057	CAAGTCGACTTCGTTCGCCATGGAATCACC
oJW058	GTCGGATCCTAAAGGGGTTAGAAAAGAGATTAGTTG
oJW059	CAAGAATTCCCAAGAAAAAGTAACAGATGG
oJW066	GATCGGCCGTCTTGTCGGCGCGATTAA
oJW067	AGAGTCGACCATGTTCGTCATCTCCTTTAA
oJW068	GAAGGATCCTAAAAAAGACGGCACCCA
oJW069	CTCGAATTCCTATGTAGATCATCTATCGGA
oQA063	CAGTCGAATTCTGAAGGGGATCAAGTGAAGC
oQA064	GATAAGGATCCCGCTGCAATATTGTGGTTA
oQA065	CAGTCGAATTCAGTATGCATAGCCGCCAGTT
oQA066	GATAAGGATCCGCAACCCGAAAGAAGCAATA
oQA077	AGGAGGAATTCTCTCCGCATTATCCTCACAAAAAAAG
oQA078	GCATCGAAACCGATATCAGCACAATCTGCTGCATTATCTGC
oQA079	GCAGATAATGCAGCAGATTGTGCTGATATCGGTTTCGATGC
oQA080	CTCCTGGTACCTGAGGAATGATTAGGAGATAGAAATTT
oQA094	CAGTCGAATTCCCTTGACGGCAGAAATAAGC
oQA095	GATAAGGATCCACGACCTCTTCGTCCACTGT

Plasmid and strain construction

*PY79 genomic was used to amplify PCR products for cloning. All marked deletion strains were confirmed by PCR.

***hag*^{HagT209C}**. To replace the *hag* gene with a mutant version (T209C), the region upstream of codon 209 of *hag* gene was PCR-amplified using the primer pair oQA77/78 and the region downstream of codon 209 of *hag* gene was PCR-amplified using the primer pair oQA79/80. The two PCR products were used as template for overlap extension PCR with primer pair oQA77/80. The amplified fragment was cut with EcoRI and KpnI and cloned into pMiniMAD cut with the same enzymes. The plasmid pQA017 was introduced to the PY79 background by single cross-over integration,

propagated in the absence selection, and plated on LB agar. Colonies were patched to identify MLS sensitive colonies and the *hag* region was sequenced to identify a strain harboring the mutation.

pJW053 [**Δ***yjbM::spec*] was generated by cloning PCR product from oJW054 and oJW055 (EcoRI-BamHI) into pKM079, then introducing PCR product from oJW052 and oJW053 (EagI-SalI).

pJW054 [**Δ***relA::spec*] was generated by cloning PCR product from oJW056 and oJW057 (EagI-SalI) into pKM079, then introducing PCR product from oJW058 and oJW059 (EcoRI-BamHI).

pJW055 [**Δ***yjbM::erm*] The SalI-BamHI spectinomycin cassette fragment of pJW053 was replaced with the SalI-BamHI fragment from pKM082 encoding the erythromycin resistance cassette (erm).

pJW058 [**Δ***relA::erm*] The SalI-BamHI spectinomycin cassette fragment of pJW054 was replaced with the SalI-BamHI fragment from pKM082 encoding the erythromycin resistance cassette (erm).

pJW063 [**Δ***ywaC::spec*] was generated by cloning PCR product from oJW066 and oJW067 (EagI-SalI) into pKM079, then introducing PCR product from oJW068 and oJW069 (EcoRI-BamHI).

pJW064 [**Δ***ywaC::erm*] The SalI-BamHI spectinomycin cassette fragment of pJW063 was replaced with the SalI-BamHI fragment from pKM082 encoding the erythromycin resistance cassette (erm)

pQA014 [*amyE::*P_{lytA}-*lacZ (cat)*] was generated in a two-way ligation with a PCR product containing the promoter region of *lytA* (primer pair oQA65/66 and PY79 genomic DNA as template) cut with EcoRI and BamHI and pDG1661 cut with the same enzymes. pDG1661 [*amyE::lacZ (cat)*] is an ectopic integration vector.

pQA015 [*amyE::*P_{hag}-*lacZ (cm)*] was generated in a two-way ligation with a PCR product containing the promoter region of *hag* (primer pair oQA63/64 and PY79 genomic DNA as template) cut with EcoRI and BamHI and pDG1661 cut with the same enzymes.

pQA017 [***hag***^{HagT209C}] was generated by overlap extension PCR. The region upstream of codon 209 of *hag* gene was PCR-amplified using the primer pair oQA77/78 and the region downstream of codon 209 of *hag* gene was PCR-amplified using the primer pair oQA79/80. The two PCR products were used as template for overlap extension PCR with primer pair oQA77/80. The amplified fragment was cut with EcoRI and KpnI and cloned into pMiniMAD cut with the same enzymes.

pQA020 [*amyE::*P_{relA}-*relA (spec)*] was generated in a two-way ligation with a PCR product containing the promoter and the coding region of *relA* (primer pair oQA94/95 and PY79 genomic DNA as template) cut with EcoRI and BamHI and pDR111 cut with the same enzymes.

REFERENCES

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