Supplemental Material

A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*

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*Running Title: Flagella mediated signal transduction

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Keywords: *Bacillus subtilis*, motility, rotation, flagellum, signal transduction, response regulator

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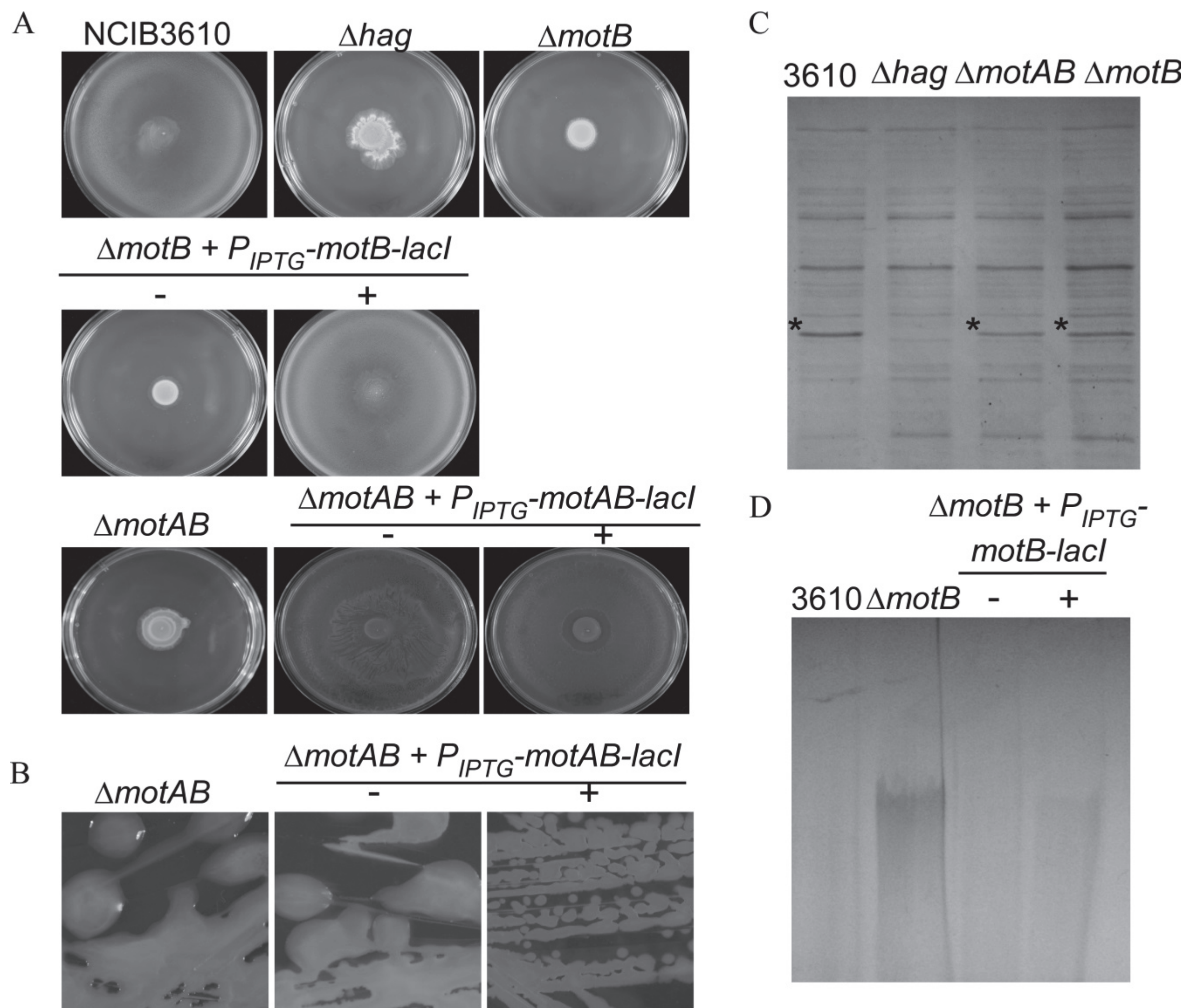

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20 **Figure S1 The Δ*motB* strain can be complemented by *motB,* but not by *motB* D^{24}A. (A)**
21 Photographs of swarm expansion assay plates taken after 6 hours incubation at 37 °C. Strains shown
22 are NCIB3610 (wild-type), Δ*hag* (DS1677), Δ*motB* (NRS3494), Δ*motB* + P_{IPTG}-*motB-lacI* (NRS3775)
23 grown in the absence and presence of 50 µM IPTG, Δ*motAB* (NRS3744) and Δ*motAB* + P_{IPTG}-*motAB-*
24 *lacI* (NRS4387) grown in the absence and presence of 50 µM IPTG **(B)** Colony morphology of
25 Δ*motAB* (NRS3744) and Δ*motAB* + P_{IPTG}-*motAB-lacI* (NRS 4387) grown overnight at 37°C on LB agar
26 in the absence and presence of 1 mM IPTG. **(C)** Coomassie stained SDS-PAGE analysis examining Hag
27 polymerisation for strains, NCIB3610 (3610), Δ*hag* (NRS2253), Δ*motAB* (NRS3744) and Δ*motB*
28 (NRS3494). The Hag protein (as identified by mass spectrometry analysis) is indicated by the
29 presence of an asterix. **(D)** SDS-PAGE of γ-PGA collected from cultures of NCIB3610, Δ*motB*
30 (NRS3494) and Δ*motB* + P_{IPTG}-*motB-lacI* (NRS3775) grown to the onset of stationary phase.
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A

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E.       MKNQAHPIIVVKRRKAKSHGAAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQIAE
B.       --------MARKKKKKHEDEHVDESWLVPYADILTLLLALFIVLYASSSIDAAKFQMLSK
                 :. *::* :..  .. ** : ***::* ::*:*:*::  *  .  ::  :::

E.       YFRTPLATAVTGGDRISNSESPIPGGGD--DYTQSQG-EVNKQPNIEELKKRMEQSRLRK
B.       SF----NEVFTGGTGVLDYSSVTPPENESDGIDEVKKEKEEKEKNKKEKEKAADQEELEN
          *       ..***  : : .*   *    :       : :  : :*: * :* :*  :*..*.:

E.       LRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFRTGSADVEPYMRDILR
B.       VKSQVEKFIKD----KKLEHQLETKMTSEGLLITIKDS---IFFDSGKATIRKEDVPLAK
         ::.:::::*:.    : *. :*: .:..*** * * **    :* :*.* :.      : :

E.       AIAP-VLNGIPNRISLSGHTDDFPYASGEKGYSNWELSADRANASRRELMVGGLDSGKVL
B.       EISNLLVINPPRNIIISGHTDNMPIKNSE-FQSNWHLSVMRAVNFMGLLIENPKLDAKVF
          *:  ::    *..* :*****::*   ..*    ***.**. **        *:      ..**:

E.       RVVGMAATMRL--SDRGPDDAVNRRISLLVLNKQAEQAILHENAESQNEPVSALEKPEVA
B.       SAKGYGEYKPVASNKTAEGRSKNRRVEVLILPRGAAET---------NEK----------
          . * .    :  .. .    : ***:..:*:* : * ::         **

E.       PQVSVPTMPSAEPR
B.       --------------
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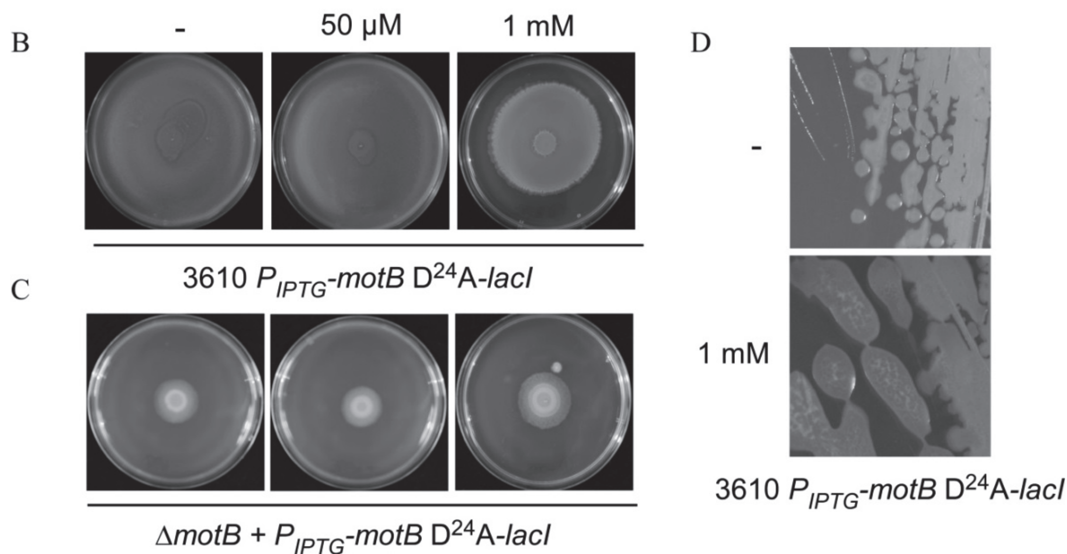

Figure S2 Characterisation of the *motB* D^{24}A mutation. (A) Sequence alignment for the MotB proteins from *E. coli* (E.) and *B. subtilis* (B.) The conserved aspartic acid residue is shown in bold and underlined text. **(B)** and **(C)** Photographs of swarm expansion assay plates taken after 6 hours incubation at 37 °C. Strains shown are: **(B)** Δ*motB* + P_{IPTG}-*motB* D^{24}A-*lacI* (NRS3867) grown in the absence and presence of 50 µM or 1mM IPTG and **(C)** P_{IPTG}-*motB* D^{24}A-*lacI* (NRS3868) grown in the absence and presence of 50 µM or 1mM IPTG. **(D)** Colony morphology of P_{IPTG}-*motB*-D^{24}A-*lacI* (NRS3868) grown on LB agar in the absence and presence of 1 mM IPTG.

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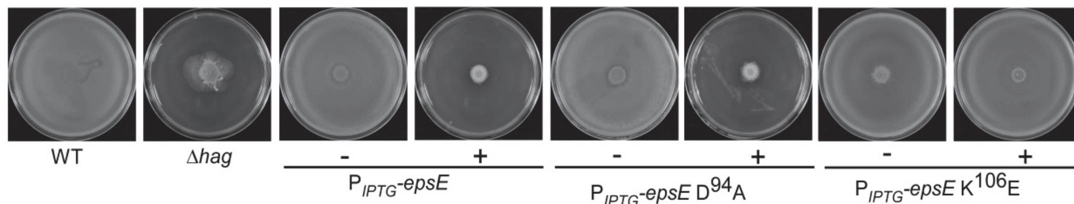

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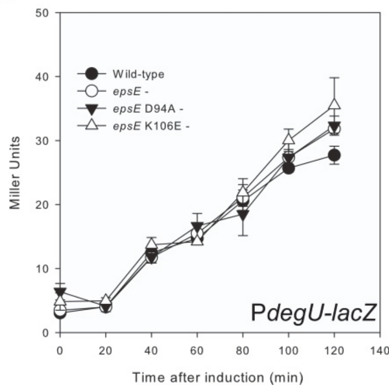

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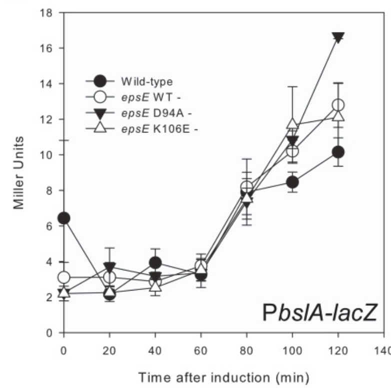

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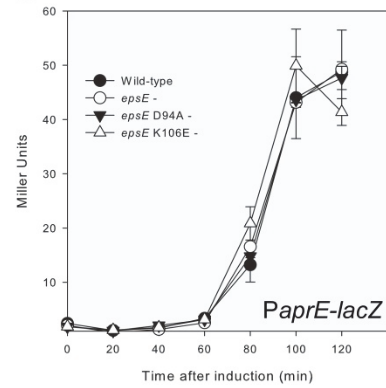

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46 **Figure S3 The clutch function of EpsE stops flagellar motility and results in γ-PGA biosynthesis. (A)**
47 Swarm expansion assay for the wild-type (WT, NCIB3610), Δ*hag* (DS1677) and P_{IPTG}-*epsE* (NRS4085),
48 P_{IPTG}-*epsE*-D^{94}A (NRS4388) and P_{IPTG}-*epsE*-K^{106}E (NRS4389) in the absence and presence of 1mM IPTG.
49 Photographs of plates were taken after 5 hours incubation at 37 °C. **(B)**, **(C)** and **(D)** β-galactosidase
50 assays of strains carrying the P*degU-lacZ*, P*bslA-lacZ* or P*aprE-lacZ* transcriptional reporter fusions,
51 respectively. Data shown are negative controls grown in the absence of IPTG (see Figure 5). Cells
52 were grown to 0.5 OD_{600}. Strains shown are **(A)** WT (wild-type NRS4351), *epsE* + (P_{IPTG}-*epsE-lacI*
53 (NRS4374)), *epsE* D^{94}A + (P_{IPTG}-*epsE*-D^{94}A-*lacI* (NRS4392)) and *epsE* K^{106}E (P_{IPTG}-*epsE*-K^{106}E-*lacI*
54 (NRS4394)). **(B)** wild-type (NRS2052), P_{IPTG}-*epsE* (NRS4405), P_{IPTG}-*epsE*-D^{94}A (NRS4406) and P_{IPTG}-*epsE*-
55 K^{106}E (NRS4407). **(C)** wild-type (NRS1561), P_{IPTG}-*epsE* (NRS4345), P_{IPTG}-*epsE*-D^{94}A (NRS4393) and P_{IPTG}-
56 *epsE*-K^{106}E (NRS4395). Data shown in parts **(B)**, **(C)** and **(D)** are plotted as the average of at least 3
57 independent replicates. Error bars represent standard error of the mean.

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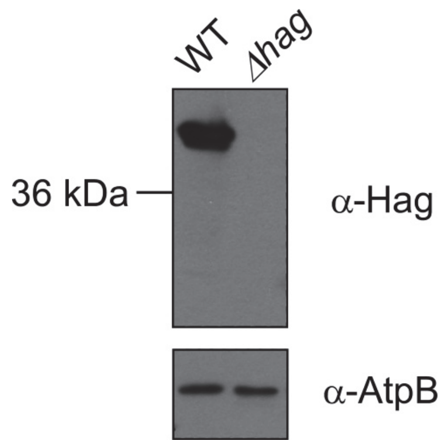

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62 **Figure S4 The Hag antibody is highly specific.** Western blot of cellular protein fractions collected
63 from WT (wild-type, NCIB3610) and Δ*hag* strain (DS1677) probed with anti-Hag and anti-AtpB as a
64 loading control.

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67 **Movie S1 Live cell microscopy of motile *Bacillus subtilis*.** Movie of the P*degU-lacZ* strain (NRS451)
68 grown in the absence of antibodies captured 5 minutes after the start of the time-course shown in
69 Fig. 7B. Movies were acquired using phase contrast, 40 X objective with the Zen high speed digital
70 recorder (Zeiss) and are shown at 5 frames per second. Movie files were created with QuickTime
71 Player v10.1. A static image from the data set is shown in Figure 7A.
72
73
74 **Movie S2 Tangling of flagella with a Hag antibody.** Movie of the P*degU-lacZ* strain (NRS451)
75 captured 5 minutes after the addition of anti-Hag antibody to the culture. Movies were acquired
76 using phase contrast, 40 X objective with the Zen high speed digital recorder (Zeiss) and are shown
77 at 5 frames per second. Movie files were created with QuickTime Player v10.1. A static image from
78 the data set is shown in Figure 7A.
79
80 **Movie S3 Live cell microscopy of motile *Bacillus subtilis*.** Movie of the P*degU-lacZ* strain (NRS451)
81 grown in the absence of antibodies captured 30 minutes after the start of the time-course shown in
82 Fig. 7B. Movies were acquired using phase contrast, 40 X objective with the Zen high speed digital
83 recorder (Zeiss) and are shown at 5 frames per second. Movie files were created with QuickTime
84 Player v10.1.
85
86 **Movie S4 Live cell microscopy of motile Bacillus subtilis grown with pre-immune sera.** Movie of the
87 P*degU-lacZ* strain (NRS451) captured 30 minutes after the addition of pre-immune sera 1 to the
88 culture. Movies were acquired using phase contrast, 40 X objective with the Zen high speed digital
89 recorder (Zeiss) and are shown at 5 frames per second. Movie files were created with QuickTime
90 Player v10.1.
91
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93

94 **Supplemental Experimental Procedures.**

95 **Western Blot.**

96 Cellular proteins were extracted as for whole cell analysis of Hag. 10 µg of cellular proteins were
97 separated by SDS-PAGE prior to transfer onto PVDF membrane (Millipore) by electroblotting.
98 Antibodies raised against Hag (a kind gift from Prof. Kürsad Turgay) were used at 1:40,000, anti-AtpB
99 (Abcam) was used at 1:10,000 and goat anti-rabbit or goat anti-mouse (both from Pierce) HRP-
100 conjugated secondary antibodies were used at 1:5,000.

101 **Plasmid Construction.**

102 **Construction of plasmid pNW680.** Plasmid pNW1045, used to introduce the *motB* coding region
103 under the control of the IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a
104 derivative of pDR111 (Britton *et al.*, 2002). The coding region of *motB* (including ribosome binding
105 site) was amplified from *B. subtilis* 3610 chromosomal DNA with primers NSW1312 and NSW1313.
106 The PCR product was digested with HindIII and SphI and cloned into pDR111 cut the same.

107 **Construction of plasmid pNW703.** Plasmid pNW703, used to disrupt the *degU* locus, contains an
108 internal region of DNA from the *degU* coding region and a kanamycin resistance cassette. To
109 construct this plasmid the internal region of *degU* was cut from pBL204 (Stanley & Lazazzera, 2005)
110 using BamHI and EcoRI and ligated into pUC19 (Vieira & Messing, 1982) also digested with BamHI
111 and EcoRI creating plasmid pNW701. The kanamycin resistance cassette was cut from plasmid
112 pDG783 (Guerout-Fleury *et al.*, 1995) using SphI and XbaI and ligated into pNW701 also digested
113 with SphI and XbaI creating pNW703.

114 **Construction of pNW1029.** Plasmid pNW1029, used to introduce the coding region of motAB under
115 the control of the IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a derivative of
116 pDR111 (Britton *et al.*, 2002). The coding region of *motAB* (including ribosome binding site) was
117 amplified from *B. subtilis* 3610 chromosomal DNA with primers NSW969 and NSW1313. The PCR
118 product was digested with SalI and SphI and cloned into pDR111 cut the same.

119 **Construction of pNW1044.** Plasmid pNW1044, used to introduce the *epsE* coding region under the
120 control of the IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a derivative of
121 pDR111 (Britton *et al.*, 2002). The coding region of *epsE* (including ribosome binding site) was
122 amplified from *B. subtilis* 3610 chromosomal DNA with primers NSW1602 and NSW1603. The PCR
123 product was digested with SalI and SphI and cloned into pDR111 cut the same.

124 **Construction of pNW1045.** Plasmid pNW1045, used to introduce a P*degU-lacZ* transcriptional
125 reporter fusion at the non-essential *thrC* locus, is a derivative of pDG1663 (Guerout-Fleury *et al.*,
126 1996). The promoter region of *degU* was amplified from *B. subtilis* NCIB3610 chromosomal DNA with
127 primers NSW404 and NSW405. The PCR product was digested with EcoRI and HindIII and cloned into
128 pDG1663 cut the same.

129 **Construction of pNW1047.** Plasmid pNW1047, used to introduce *epsE* $D^{94}A$ under control of the
130 IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a derivative of pNW1044. The
131 D94A mutation was introduced to pNW1044 by site-directed mutagenesis with primers NSW1608
132 and NSW1609, using PCR amplification conditions calculated according to the Stratagene
133 Quikchange manual using KOD Hot Start DNA polymerase (Novagen). PCR products were then
134 treated with Dpn*I* and transformed into competent *E. coli* MC1061 cells. Plasmid pNW1048 for *epsE*
135 $K^{106}E$ was constructed in an identical manner using primers NSW1610 and NSW1611.

136 **Construction of pNW1060.** Plasmid pNW1060, used to introduce *motB* $D^{24}A$ under the control of an
137 IPTG-inducible promoter $P_{hy\text{-}spank}$ at the non-essential *amyE* locus, is a derivative of pNW680. Briefly,
138 pNW680 was cut with HindIII and SphI to excise *motB*, which was ligated into pUC19 cut the same to
139 create plasmid pNW1058. pNW1058 was used as template DNA in a site-directed mutagenesis PCR
140 reaction (Stratagene ™) with primers NSW1011 and NSW1012 to introduce the $D^{24}A$ point mutation
141 and yield plasmid pNW1059. pNW1059 was then cut with HindIII and SphI to excise *motB* $D^{24}A$,
142 which was ligated into pDR111 to create pNW1060.

143

144 **Table S1 Strains used in this study.**

Strain	Relevant Genotype[a]	Source[b]
NCIB 3610	Prototroph	B.G.S.C.
168	*trpC2*	B.G.S.C.
JH642	*trpC2 pheA1*	(Perego & Hoch, 1988)
RL2420	PY79 pIC333	(Kearns *et al.*, 2004)
DS1677	3610 Δ*hag*	Kind gift D. Kearns
NRS1136	JH642 *degS::cml*	(Verhamme *et al.*, 2007)
NRS1154	JH642 *pgdS::pBL141(cml)*	(Stanley & Lazazzera, 2005)
NRS1314	3610 *degU::pBL204 (cml)*	(Verhamme *et al.*, 2007)
NRS1325	3610 *degU::pBL204 (cml) amyE::*$P_{hy\text{-}spank}$*-degU32-hy-lacI (spc)*	(Verhamme *et al.*, 2007)
NRS1433	168 *pgsB::spc*	(Stanley & Lazazzera, 2005)
NRS1534	168 *thrC::*P*aprE-lacZ (mls)*	(Verhamme *et al.*, 2007)
NRS1561	3610 *thrC::*P*aprE-lacZ (mls)*	(Verhamme *et al.*, 2007)
NRS2051	JH642 *thrC::PbslA-lacZ (mls)*	(Verhamme *et al.*, 2009)
NRS2052	3610 *thrC::PbslA-lacZ (mls)*	(Verhamme *et al.*, 2009)
NRS3347	3610 *pgdS::cml*	NRS1154→NCIB3610
NRS3348	3610 Δ*motB* + *pgdS::cml*	NRS1154→NRS3494
NRS3374	168 *degU::*pNW703 *(kan)*	pNW703 →168
NRS3433	3610 *pgsB::spc*	SPP1 NRS1433 →NCIB3610
NRS3434	3610 Δ*motB* + *pgsB::spc*	SPP1 NRS1433 → NRS3494
NRS3494	3610 Δ*motB*	pNW654 → NCIB3610
NRS3514	168 *amyE::*$P_{hy\text{-}spank}$*-motB-lacI (spc)*	pNW680 → 168
NRS3744	3610 Δ*motAB*	pNW1021→ NCIB3610
NRS3764	3610 *pgsB::spc* + *degU::kan*	SPP1 NRS3374 →NRS3433
NRS3769	168 *amyE::*$P_{hy\text{-}spank}$*-motAB-lacI (spc)*	pNW1029 → 168
NRS3770	3610 *pgsB::spc* + *degU::kan amyE::*$P_{hy\text{-}spank}$*-degU32-hy-lacI (cml)*	SPP1 NRS1268 →NRS3764
NRS3775	3610 Δ*motB* + $P_{hy\text{-}spank}$*-motB-lacI (spc)*	SPP1 NRS3514 →NRS3494
NRS3866	168 $P_{hy\text{-}spank}$*-motB*D^{24}A*-lacI (spc)*	pNW1051→168
NRS3867	3610 Δ*motB* + *amyE::*$P_{hy\text{-}spank}$*-motB*D^{24}A*-lacI (spc)*	SPP1 NRS3866 →NRS3494
NRS3868	3610 *amyE::* $P_{hy\text{-}spank}$*-motB*D^{24}A*-lacI (spc)*	SPP1 NRS3866 →NCIB3610
NRS3870	3610 Δ*motB* + *amyE::*$P_{hy\text{-}spank}$*-motB*D^{24}A*-lacI (spc) thrC::*P*aprE-lacZ (mls)*	SPP1 NRS1534 →NRS3867
NRS4083	168 *amyE::*$P_{hy\text{-}spank}$*-epsE-lacI (spc)*	pNW1044 →168
NRS4084	168 *thrC::*P*degU-lacZ (mls)*	pNW1045 →168
NRS4085	3610 *amyE::*$P_{hy\text{-}spank}$*-epsE-lacI (spc)*	SPP1 NRS4083 →NCIB3610
NRS4093	3610 Δ*motAB* + *thrC::*P*aprE-lacZ (mls)*	SPP1 NRS1534 →NRS3744
NRS4345	3610 *amyE::*$P_{hy\text{-}spank}$*-epsE-lacI (spc) thrC::*P*aprE-lacZ (mls)*	SPP1 NRS1534 →NRS4085
NRS4351	3610 *thrC::*P*degU-lacZ (mls)*	SPP1 NRS4084 →NCIB3610
NRS4353	3610 Δ*motB* + *thrC::*P*degU-lacZ (mls)*	SPP1 NRS4084 →NRS3494
NRS4354	3610 Δ*motAB thrC::*P*degU-lacZ (mls)*	SPP1 NRS4084 →NRS3744
NRS4373	3610 *degU::cml* + *thrC::*P*degU-lacZ (mls)*	SPP1 NRS4084 →NRS1314
NRS4374	3610 *amyE::*$P_{hy\text{-}spank}$*-epsE-lacI (spc) thrC::*P*degU-lacZ (mls)*	SPP1 NRS4084 →NRS4085

Strain	Relevant Genotype[a]	Source[b]
NRS4385	168 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K^{106}E-*lacI (spc)*	pNW1048 →168
NRS4386	168 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc)*	pNW1047 →168
NRS4387	3610 Δ*motAB* + *amyE::*$P_{hy\text{-}spank}$-*motAB-lacI*	SPP1 NRS3469 →NRS3744
NRS4388	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc)*	SPP1 NRS4386 →NCIB3610
NRS4389	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K^{106}E-*lacI (spc)*	SPP1 NRS4385 →NCIB3610
NRS4392	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc) + thrC::PdegU-lacZ (mls)*	SPP1 NRS4084 →NRS4388
NRS4393	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc) + thrC::PaprE-lacZ (mls)*	SPP1 NRS1534 →NRS4388
NRS4394	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K106E-*lacI (spc) + thrC::PdegU-lacZ (mls)*	SPP1 NRS4084 →NRS4389
NRS4395	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K106E-*lacI (spc) thrC::PaprE-lacZ (mls)*	SPP1 NRS1534 →NRS4389
NRS4396	3610 Δ*motB* + *thrC::*P*degU-lacZ (mls) amyE::*$P_{hy\text{-}spank}$-*motB-lacI (spc)*	SPP1 NRS3514 →NRS4353
NRS4397	3610 Δ*motB* + *thrC::*P*degU-lacZ (mls) amyE::*$P_{hy\text{-}spank}$-*motB*-D^{24}*A-lacI (spc)*	SPP1 NRS3866 →NRS4353
NRS4398	3610 Δ*motB* + *degS::cml*	SPP1 NRS1136 →NRS3494
NRS4399	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE-lacI (spc) + thrC::PaprE-lacZ (mls)*	SPP1 NRS1136 →NRS4345
NRS4400	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc) + thrC::PaprE-lacZ (mls)*	SPP1 NRS1136 →NRS4393
NRS4401	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K106E-*lacI (spc) + thrC::PdegU-lacZ (mls)*	SPP1 NRS1136 →NRS4395
NRS4405	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE-lacI (spc) + thrC::PbslA-lacZ (mls)*	SPP12051→NRS4345
NRS4406	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-D^{94}A-*lacI (spc) + thrC::PbslA-lacZ (mls)*	SPP12051→NRS4393
NRS4407	3610 *amyE::*$P_{hy\text{-}spank}$-*epsE*-K106E-*lacI (spc) + thrC::PbslA-lacZ (mls)*	SPP12051→NRS4395

145 [a] Drug resistance cassettes are indicated as follows: *kan*, kanamycin resistance; *mls*,
146 lincomycin/erythromycin resistance; *cml*, chloramphenicol resistance and *spc*, spectinomycin
147 resistance.
148 [b] The direction of strain construction is indicated with DNA or phage (SPP1) (→) recipient strain.
149 B.S.G.C. is the *Bacillus* genetic stock centre.

150

151 **Table S2 Plasmids used in this study.**

Plasmid	Relevant Genotype [a]	Source
pDR111	*amyE* integration plasmid *(spc)*	(Britton *et al.*, 2002)
pMAD	In-frame markerless deletion plasmid	(Arnaud *et al.*, 2004)
pDG1663	*lacZ* reporter fusion plasmid, *thrC* integration	(Guerout-Fleury *et al.*, 1996)
pUC19	Cloning plasmid	(Vieira & Messing, 1982)
pBL204	pUC19 containing a *cml* resistance cassette	(Stanley & Lazazzera, 2005)
pNW651	Region upstream of *motB* in pUC19	This study
pNW652	Region downstream of *motB* in pUC19	This study
pNW653	Δ*motB* in pUC19	This study
pNW654	Δ*motB* in pMAD	This study
pNW680	*motB* in pDR111	This study
pNW701	pUC19 containing internal region of *degU*	This study
pNW703	Kanamycin resistance cassette in an internal region of *degU* coding region	This study
pNW1019	Δ*motAB* in pUC19	This study
pNW1021	Δ*motAB* in pMAD	This study
pNW1029	*motAB* in pDR111	This study
pNW1044	*epsE* in pDR111	This study
pNW1045	P*degU* in pDG1663	This study
pNW1047	*epsE* $D^{94}A$ in pDR111	This study
pNW1048	*epsE* $K^{106}E$ in pDR111	This study
pNW1058	*motB* in pUC19	This study
pNW1059	*motB* $D^{24}A$ in pUC19	This study
pNW1060	*motB* $D^{24}A$ in pDR111	This study

152 [a] Drug resistance cassettes are indicated as follows: *cml*, chloramphenicol resistance; *kan*, kanamycin
153 resistance, and *spc*, spectinomycin resistance.
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Table S3 Primers used in this study.

Primer	**Target**	**Sequence** [a]	**Position** [b]
NSW404	*degU*	GCATGAATTCGGCCTGATTCCAACTTTAA	-487→-468
NSW405	*degU*	GCATAAGCTTCTGATGGTCGTCGATAAT	+26→+39
NSW874	*motB*	GTACGGATCCATTGAGGATGTAGATGATGC	-480→-460
NSW875	*motB*	ATGCTCTAGAGTACAGCACAATAAACAATGC	+77→+98
NSW876	*motB*	GCATTCTAGAGTTGAAGTTCTCATTTTGCCG	+700→+721
NSW877	*motB*	GCATGTCGACCATCGCTCCAACATACACC	+1500→+1519
NSW965	*motA*	GCTAGGATCCTTGAGGATGAAATGACCGATCTGC	-479→-156
NSW966	*motA*	GCTATCAGACGAAGTTTTATCCATAGTTTTCACC	-10→+16
NSW969	*motA*	GCTAGTCGACAGACAAGCTAGTAAAAAAGGATTTGG	-66→-9
NSW1011	*motB*	TCATGGCTCGTTCCTTACGCCG**C**CATCCTTACTCTTCTCCTG	+49→+91
NSW1012	*motB*	CAGGAGAAGAGTAAGGATG**G**CGGCGTAAGGAACGAGCCATGA	+49→+91
NSW1312	*motB*	GCATAAGCTTGCAGAACAAGGAGAGGCGCAAAAT	-30→-6
NSW1313	*motB*	GCATGCATGCCTATTTTTCATTTGTTTCCGCTGCGC	+880→+904
NSW1474	*pgsB*	CTGTTAACCCAGATTATCAAATC	+331→+354
NSW1475	*pgsB*	CTGCGCGGCAGTTCATGATGAT	+889→+868
NSW1602	*epsE*	AGGAGGTCGACAAAGGAGAAAAGCGTATGAACTCAG	-16→-1
NSW1603	*epsE*	CTCCTGCATGCTGGCTGCTATTCATGCTTGACAAG	+820→+843
NSW1604	*pgdS*	GGAGACGGCCAAATGGTTC	+370→+388
NSW1605	*pgdS*	GCAAGCCGGTCAGAAAAAG	+778→+796
NSW1608	*epsE*	CGCACGTCAG**G**CCGGAGATGACCTTTCG	+270→+298
NSW1609	*epsE*	CGAAAGGTCATCTCCG**G**CCTGACGTGCG	+270→+298
NSW1610	*epsE*	CCGCGCCGTCTGGAA**G**AGCAGGTCGCGTTTTTA	+301→+333
NSW1611	*epsE*	TAAAAACGCGACCTTCT**C**TTCCAGACGGCGCGG	+301→+333
DEN5	*16S rRNA*	TCACGRCACGAGCTGACGAC	
DEN7	*16S rRNA*	ACTCCTACGGGAGGCAGC	

[a] Underlined sequences indicate endonuclease restriction cut sites. Bold sequences represent base pairs mutated by site-directed mutagenesis.
[b] Position of primer is indicated in relation to the translational start site (noted as +1) of the named gene.

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