Figure S1

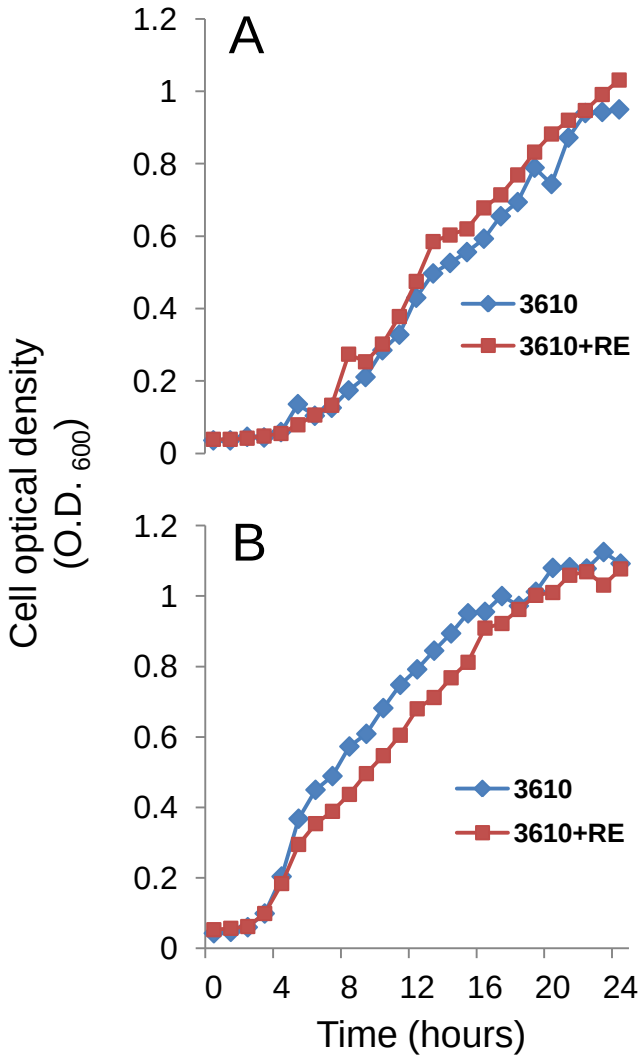

A
B
1.2
1
0.8
0.6
0.4
0.2
0
3610
3610+RE
Cell optical density
(O.D. 600)
0
4
8
12
16
20
24
Time (hours)

Fig. S1. Growth curve of *B. subtilis* wild type cells in the minimal medium and in LB medium. 3610 cells were inoculated either in the minimal medium (panel A) or LB medium (panel B) in 24-well plates and grown at 37°C with shaking. Cell optical density of the cultures was measured periodically during a period of 24 hours. RE represents addition of 1% of the root exudate in the medium.

Figure S2

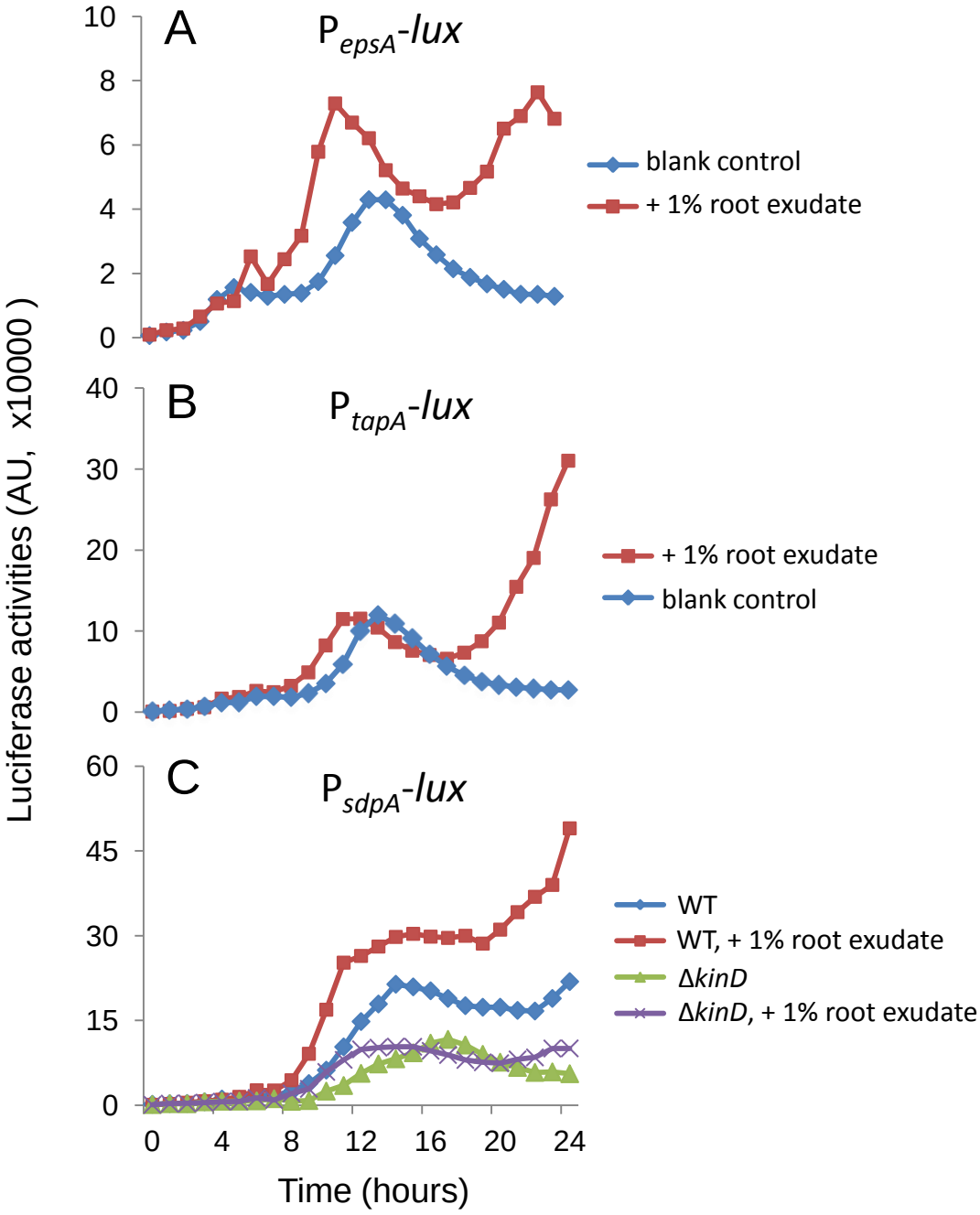
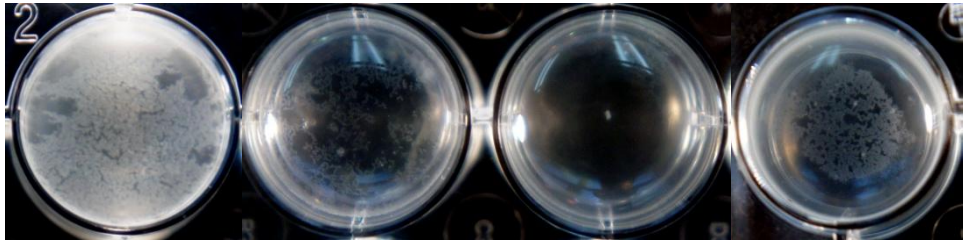

A
P epsA -lux
10
8
6
4
2
0
blank control
+ 1% root exudate
B
P tapA -lux
40
30
20
10
0
+ 1% root exudate
blank control
C
P sdpA -lux
60
45
30
15
0
WT
WT, + 1% root exudate
ΔkinD
ΔkinD, + 1% root exudate
0
4
8
12
16
20
24
Time (hours)
Luciferase activities (AU, x10000)

Fig. S2. Tomato root exudates induce expression of the matrix genes and the *sdpABC* operon in the minimal medium. Panels A and B show luciferase activities for the two wild type reporter strains harboring either P_{epsA}-*lux* (strain ALM89 in A) or P_{tapA}-*lux* (strain ALM90 in B) that were grown in the minimal medium with (squares in red) or without (diamonds in blue) addition of 1% root exudate. Panel C shows luciferase activities of the wild type (CY136) and the Δ*kinD* mutant (CY137) cells that harbored the P_{sdpA}-*lux* reporter fusion in the presence (squares in red for WT and crosses in purple for Δ*kinD*) or absence (diamonds in blue for WT and triangles in green for Δ*kinD*) of 1% root exudate. The luciferase activities were presented in arbitrary units (AU).

Figure S3



3610
+ 1% root exudate

3610

ΔkinD
+1% root exudate

ΔkinD

Fig. S3. Pellicle induction of the wild type but not the Δ*kinD* mutant cells in response to tomato root exudates in the minimal medium. Cells were inoculated to the minimal medium in 6-well plates and were incubated at 22°C for three days. Wild type cells (3610) formed pellicles in the minimal medium with the addition of 1% root exudates whereas the Δ*kinD* mutant cells (RL1927) did not respond to the addition of root exudates in pellicle induction.

Figure S4

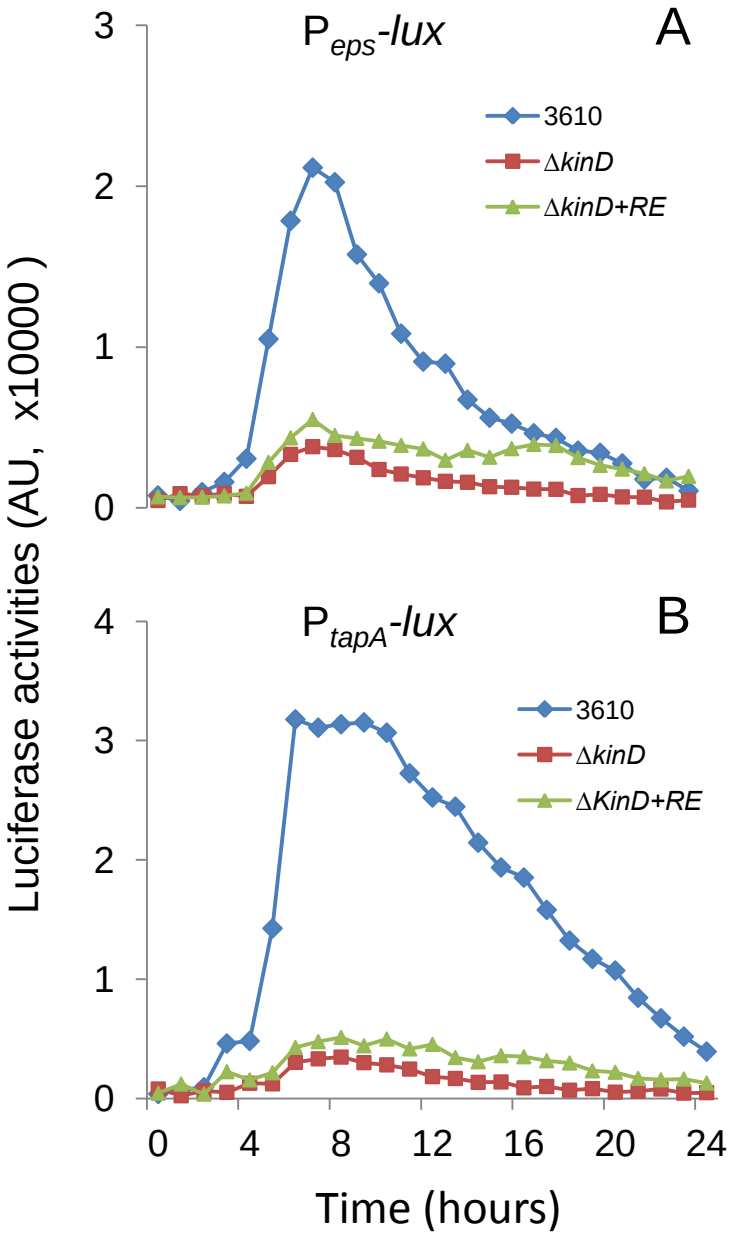

P eps -lux
A
3610
ΔkinD
ΔkinD+RE
P tapA -lux
B
3610
ΔkinD
ΔKinD+RE
Luciferase activities (AU, x10000)
Time (hours)
0
1
2
3
4
0
4
8
12
16
20
24

Fig. S4. Response of the matrix genes to the tomato root exudate depend on KinD. Panels A and B show luciferase activities for the two wild type reporter strains harboring either P_{epsA}-*lux* (strain ALM89 in A) or P_{tapA}-*lux* (strain ALM90 in B) and the two Δ*kinD* mutant strains harboring the same reporters (strain CY414 in A and strain CY415 in B) that were grown in LB shaking culture. Symbols are as follows: 3610, - root exudate (diamonds in blue); Δ*kinD*, - root exudate (squares in red); Δ*kinD*, + root exudate (triangles in green). RE represents addition of 1% of the root exudate in the medium. The luciferase activities were presented in arbitrary units (AU).

Figure S5

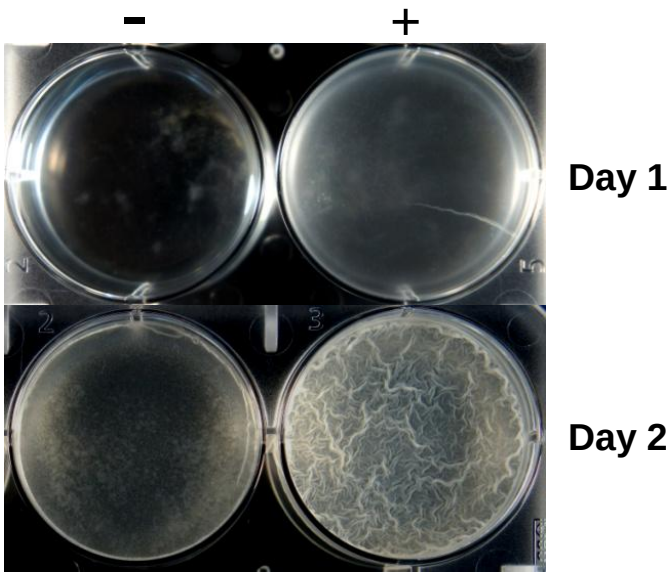


Fig. S5. $_{L}$-Malic acid stimulates early pellicle formation by *B. subtilis* in MSgg. 3610 cells were inoculated into 9-ml of MSgg liquid medium in a 6-well plate. $_{L}$-malic acid was added at a final concentration of 5 mM in one sample and absent from the other. Cell samples were incubated at 22°C. Images of the pellicles were taken 48 h and 72 h after inoculation.

Supplemental methods

Strain constructions. To construct the *kinD* mutant strains that bear either the P_{epsA}-*lux* or the P_{tapA}-*lux* reporter fusion, the DNA fragment containing the insertional deletion of *kinD* (Δ*kinD*::mls) was introduced into ALM89 and ALM90 by SSP1 phage-mediated transduction, resulting in strains CY414 and CY415, respectively. To construct strains that express fusion proteins between GFP and the wild type KinD or the CACHE domain mutant of KinD, the promoter sequence and the coding region of the *kinD* gene were amplified by PCR using the chromosomal DNA of CY185 or CY186 as the template. Note that CY186 contains the CACHE domain point mutations in the *kinD* gene. PCR products (P_{kinD} -*kinD*^{Wt} and P_{kinD} -*kinD*^{Mut}) were digested by EcoRI/BamHI and ligated into the plasmid pYC121 pre-digested with the same two restriction enzymes, generating the recombinant plasmids pCY393 and pCY394, respectively. The plasmids pCY393 and pCY394 were first introduced into PY79 by transformation. Integration of P_{kinD} -*kinD-gfp* (wt or mut) via double crossover recombination at the *amyE* site was carefully verified. The DNA fragment containing *amyE*:: P_{kinD} -*kinD-gfp* (wt or mut) was introduced into RL4569 (Δ*kinD*::*mls*) by SSP1 phage-mediated transduction, generating strains CY 416 and CY417.

Immunoblot analysis. 20-ml of log phase cells ($O.D._{600}$=1.2) were harvested and washed with 5 ml of cold phosphate buffer (25 mM sodium phosphate buffer, pH7.2, 200 mM NaCl, 10% glycerol). Cells were resuspended in 2 ml of the same phosphate buffer (supplemented with 200 μg/ml freshly made lysozyme, 1 mM DTT, 1 mM PMSF) and incubated on ice for 30 min. Samples were centrifuged and the pellets containing

cell debris and membrane fractions were resuspended with 2 ml of cold phosphate buffer supplemented with 6M urea. Treated samples were centrifuged again. The supernatants from the cytoplasmic fraction and the urea-treated membrane fraction were combined and were applied in western immunoblot assays. Western immunoblot was done similarly to a protocol described previously (Chai *et al.*, 2009). The fusion proteins were detected by commercially available antibodies against GFP (Abcam, MA, USA).

Table S1. An HHpred search based on the CACHE domain of KinD.

No.	Hit	Feature	Prob	P-value	Query HMM	Template HMM
1	3fos_A	sporulation histidine kinase	100.0	0	1-214	1-214
2	3lif_A	putative diguanylate cyclase	99.9	7.3E-28	13-186	5-193
3	3c8c_A	methyl-accepting chemotaxis protein	99.9	7.7E-27	7-183	2-183
4	3e4o_A	C4-dicarboxylate sensor protein DctB	99.8	4.7E-23	4-188	43-233
5	3lic_A	sensor protein with PDC fold	99.8	4.7E-23	4-176	7-197
6	3li9_A	hypothetical histidine kinase	99.8	1.2E-21	4-184	7-213
7	3lib_A	hypothetical histidine kinase	99.8	4.0E-21	4-192	6-220
8	3by9_A	histidine kinase sensor domain	99.7	5.4E-21	2-188	3-192
9	3lid_A	putative sensory BOX/ggdef protein	99.7	2.0E-20	5-191	9-220
10	1p0z_A	sensor kinase CitA	99.2	9.0E-15	27-149	6-128
11	3cwf_A	alkaline phosphatase	98.0	4.7E-09	22-149	7-117
12	3b42_A	methyl-accepting protein	97.7	5.8E-08	5-151	1-131
13	2qkp_A	uncharacterized protein	97.5	2.6E-08	54-152	18-136
14	3b47_A	methyl-accepting protein	97.4	2.0E-07	5-147	1-126
***** 15	3c38_A	autoinducer 2 sensor kinase LuxQ	97.4	6.8E-08	4-181	11-198
16	3luq_A	sensor protein with PAS domain	97.2	1.3E-07	55-146	3-113
17	2gj3_A	nitrogen fixation regulator	96.8	3.7E-07	54-146	4-116
18	3lyx_A	sensory BOX/ggdef domain	96.6	4.3E-07	53-146	5-118
19	3icy_A	sensor protein; sensory kinase	96.5	1.1E-06	54-146	3-117
20	3cax_A	uncharacterized protein	96.4	1.0E-06	54-151	238-349
21	2z6d_A	phototropin-2; PAS-fold	95.7	2.1E-06	51-146	2-120
22	3ewk_A	sensor protein; PAS domain	95.7	3.0E-06	70-175	2-137

* The star indicates the hit of LuxQ based on HHpred search of the CACHE domain of KinD.

Table S2. Small chemical molecules identified in tomato root exudates.

categories	compounds
amino acid	glycine alanine aspartic acid arginine glutamic acid valine threonine trimethylglycine
organic acid	citric acid malic acid succinic acid fumaric acid γ-aminobutanoic acid
sugar	glucose fructose maltose xylose ribose inositol

Table S3. Strains used in this study.

name	genetype	reference
E.coli		
DH5α	an *E. coli* strain used for molecular cloning	Invitrogen
RL1936	DH5α derivative containing the plasmid pDG780, Amp^R, Kan^R	Losick lab collection
YC311	DH5α derivative containing the plasmid pYC121, Amp^R, Cm^R	(Chai *et al.*, 2008)
YC317	DH5α derivative containing the plasmid pYC127, Amp^R, Cm^R	(Chai et al., 2008)
CY393	DH5α derivative containing the plasmid pCY393, Amp^R, Cm^R	This study
CY394	DH5α derivative containing the plasmid pCY394, Amp^R, Cm^R	This study
YC433	DH5α derivative containing the plasmid pYC240, Amp^R, Spc^R	This study
YC434	DH5α derivative containing the plasmid pYC241, Amp^R, Spc^R	This study
RL1927	DH5α derivative containing the plasmid pDG1662, Spc^R	(Guérout-Fleury *et al.*, 1996)
TMN387	DH5α derivative containing the plasmid pMKate2, Amp^R	a gift of Norman T
B. subtilis		
PY79	laboratory strain used as a host for transformation	
3610	undomesticated wild strain capable of forming robust biofilms	(Branda *et al.*, 2001)
ALM89	*sacA*:: P_{epsA}-*lux* in 3610, Cm^R	(McLoon *et al.*, 2011
ALM90	*sacA*:: P_{tapA}-*lux* in 3610, Cm^R	(McLoon et al., 2011
CY1	Δ*epsA-O* in 3610, Kan^R	This study
CY49	*amyE*::$P_{hyspank}$-*mKate2* in 3610, Cm^R	This study
CY78	Δ*kinD*, *amyE*:: *kinD*^{wt} in 3610, Mls^R, Spc^R, Cm^R	This study
CY79	Δ*kinD*, *amyE*:: *kinD*^{mut} in 3610, Mls^R, Spc^R, Cm^R	This study
CY126	Δ*kinC*, Δ*kinD, amyE*::$P_{hyspank}$-*mKate2* in 3610, Mls^R, tet^R, Cm^R	This study
CY127	Δ*kinD*, *amyE*::$P_{hyspank}$-*mKate2* in 3610, Mls^R, Cm^R	This study
CY136	*sacA*:: P_{sdpA}-*lux* in 3610, Cm^R	This study
CY137	Δ*kinD*, *sacA*:: P_{sdpA}-*lux* in 3610, Mls^R, Cm^R	This study
CY185	Δ*kinD*, *amyE*::*kinD*^{wt}, *sacA*:: P_{sdpA}-*lux* in 3610, Mls^R, Spc^R, Cm^R	This study
CY186	Δ*kinD*, *amyE*::*kinD*^{mut}, *sacA*:: P_{sdpA}-*lux* in 3610, Mls^R, Spc^R, Cm^R	This study
CY189	Δ*kinD*, *amyE*::$P_{hyspank}$-*mKate2,* 317°Ω*amyE*::*kinD*^{wt} in 3610, Mls^R, Cm^R, Spc^R	This study
CY190	Δ*kinD*, *amyE*::$P_{hyspank}$-*mKate2,* 317°Ω*amyE*::*kinD*^{mut} in 3610, Mls^R, Cm^R,Spc^R	This study
CY204	Δ*kinA*, *amyE*::$P_{hyspank}$-*mKate2* in 3610, Mls^R, Cm^R	This study
CY205	Δ*kinB*, *amyE*::$P_{hyspank}$-*mKate2* in 3610, Kan^R, Cm^R	This study
CY206	Δ*kinA*, Δ*kinB, amyE*::$P_{hyspank}$-*mKate2* in 3610, Mls^R, Kan^R, Cm^R	This study
CY207	Δ*kinC*, *amyE*::$P_{hyspank}$-*mKate2* in 3610, Mls^R, Cm^R	This study
CY209	Δ*epsA-O*, *amyE*::$P_{hyspank}$-*mKate2* in 3610, Kan^R, Cm^R	This study
CY414	Δ*kinD*, *sacA*:: P_{epsA}-*lux* in 3610, Mls^R, Cm^R	This study
CY415	Δ*kinD*, *sacA*:: P_{tapA}-*lux* in 3610, Mls^R, Cm^R	This study
CY416	Δ*kinD, amyE::PkinD kin*^{Wt}*-GFP,* Mls^R, Cm^R	This study
CY417	Δ*kinD, amyE::PkinD kin*^{Mut}*-GFP,* Mls^R, Cm^R	This study
RL4562	Δ*kinA* in 3610, Mls^R	(McLoon et al., 2011)
RL4563	Δ*kinB* in 3610, Kan^R	(McLoon et al., 2011)
RL4262	Δ*kinC* in 3610, Mls^R	(McLoon et al., 2011)
RL4552	Δ*kinD* in 3610, Tet^R	(McLoon et al., 2011)
RL4569	Δ*kinD* in 3610, Mls^R	(McLoon et al., 2011)
RL4573	Δ*kinA*, Δ*kinB* in 3610, Mls^R, Kan^R	(McLoon et al., 2011)
RL5273	Δ*kinC*, Δ*kinD* in 3610, Mls^R , Tet^R	(McLoon et al., 2011)
YC751	317°Ω *amyE*::Cm^R in PY79	This study

Table S4. Primers used in this study.

eps-KO-P1	5'-GCTGTGGCATCAAGCACATCT-3'
eps-KO-P2	5'-CAATTCGCCCTATAGTGAGTCGTAACTCATATTCTCATTCATGTAT-3'
eps-KO-P3	5'-CCAGCTTTTGTTCCCTTTAGTGAGTCCTGCTCACATGTGAGCGGAA-3'
eps-KO-P4	5'-GGTCTAGGATGAAGAGCCGCGATA-3'
kinD-F1	5'-GTACGAATTCCAGTGATTTTTCTGTCATGTCTC-3'
kinD-MF1	5'-AACCTGGCCGACCTATTAGATTCTATAAAAGCAAAGG-3'
kinD-MR1	5'-CCTTTGCTTTTATAGAATCTAATAGGTCGGCCAGGTT-3'
kinD-R1	5'-GTACGGATCCTATGATGCGGATACGGGGAGGG-3'
mkate2-F1	5'-GTACAAGCTTAAGGAGGAACTACTATGGATTCAATAGAAAAGGTAAG-3'
kinD-R2	5'-GTACGGATCCTGATGCGGATACGGGGAGGGTGA-3'
mkate2-R1	5'-GTACGGATCCTTATCTGTGCCCCAGTTTGCT-3'

Table S5. Plasmids used in this study.

Plasmid	feature	reference
pCY393	*amyE*:: P_{kinD} kin^{wt}-*gfp*, cm^R, amp^R	this study
pCY394	*amyE*:: P_{kinD} kin^{mut}-*gfp*, cm^R, amp^R	this study
pDG780	a plasmid for Campbell integration in *B. subtilis*, kan^R, amp^R	Losick lab collection
pDG1662	a plasmid for integration at *amyE* in *B. subtilis*, cm^R, amp^R	(Guérout-Fleury et al., 1996)
pMKate2	contains a gene encoding the far-red fluorescent proteins, amp^R	Evrogen
pYC121	contains a promoter-less *gfp* for *amyE* integration, cm^R, amp^R	(Chai et al., 2008)
pYC127	contains $P_{hyspank}$-*gfp*, an *amyE* integration vector, cm^R, amp^R	(Chai et al., 2008)
pYC240	contains *amyE*:: P_{kinD} -kin^{Wt}, an *amyE* integration vector, $spec^R$, amp^R	this study
pYC241	contains *amyE*:: P_{kinD} –kin^{Mut} , an *amyE* integration vector, $spec^R$, amp^R	this study

Supplemental references

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