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Supplementary Materials for

Control of potassium homeostasis is an essential function of the second messenger cyclic di-AMP in *Bacillus subtilis*

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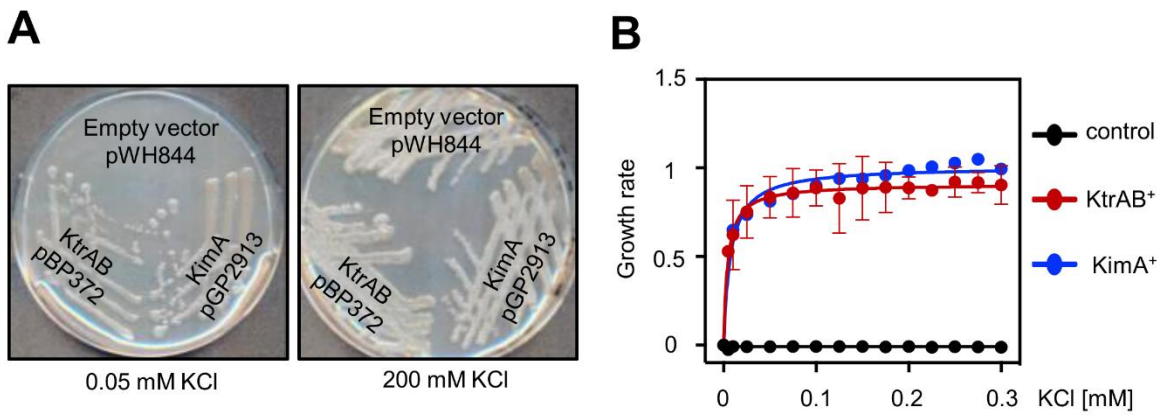

Fig. S1. Activity of *B. subtilis* K^+ transporters in *E. coli*. (**A**) Complementation assay with KimA in *E. coli* strain LB650, which lacks all native potassium transporters. LB650 was transformed with the IPTG-inducible empty vector pWH844, pBP372 (KtrAB) and pGP2913 (KimA). Cells were grown during the day in 4 ml LB-K without IPTG induction; the cells were then centrifuged, washed and resuspended in washing buffer and subsequently plated on minimal media containing 1 mM IPTG and two different potassium concentrations (low: 0.05 mM KCl; high: 200 mM KCl). (**B**) Dependence of growth rate on external K^+ concentration for *E. coli* LB650 harboring the empty vector (pWH844) or synthesizing either KtrAB (pBP372), or KimA (pGP2913) grown in minimal medium supplemented with the indicated potassium concentrations. Saturation curves were fitted according to the Michaelis-Menten equation (n = 3 experiments).

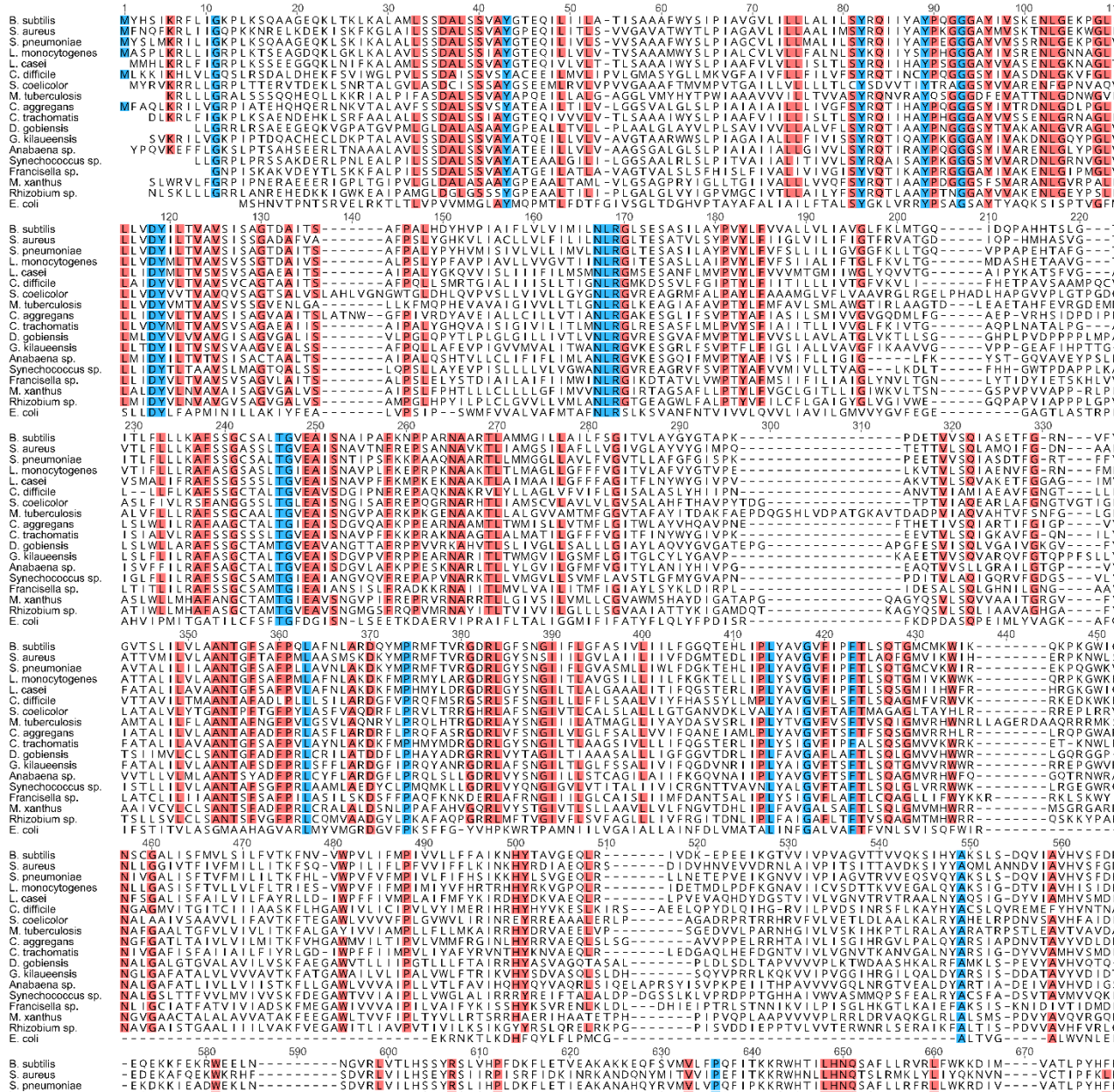

Fig. S2. Alignment of KimA homologs. ClustalW alignments of KimA from *B. subtilis* and homologs from *S. aureus*, *S. pneumoniae*, *L. monocytogenes*, *L. casei*, *C. difficile*, *S. coelicolor*, *M. tuberculosis*, *C. aggregans*, *C. trachomatis*, *D. gobiensis*, *G. kilaueensis*, *Anabaena sp.*, *Synechococcus sp.*, *Francisella sp.*, *M. xanthus*, *Rhizobium sp.*, *E.coli*. Amino acids in blue are conserved in all sequences; amino acids in red are conserved in at least 80% of the sequence.

Strain	Genotype	Reference/ Construction
168	*trpC2*	Laboratory collection
JH642	*trpC2 pheA1*	*9*
BP144	*trpC2 amyE*::(P_{kimA}-RS-*lacZ cat*)	pBP135 → 168
BP193	*trpC2 amyE*::(P_a-*lacZ cat*)	pBP164 → 168
BP610	*trpC2 disA-Strep spc*	pBP599 → 168
GHB1	*trpC2 pheA1* Δ*ktrAB*::*aphA3*	*9*
GHB12	*trpC2 pheA1* Δ*ktrD*::*tet*	*9*
GHB15	*trpC2 pheA1* ΔktrD::tet ΔktrC::spc Δ*ktrAB*::*aphA3*	*9*
GP92	*trpC2* Δ*ktrAB*::*aphA3*	GHB1 → 168
GP93	*trpC2* Δ*kimA*::*cat*	LFH → 168
GP94	*trpC2* Δ*cdaA*::*spc*	LFH-PCR → 168
GP715	*trpC2* Δ*yhcK*::*ermC* Δ*dgcW*::*cat*	LFH → GP848
GP848	*trpC2* Δ*yhcK*::*ermC*	LFH → 168
GP850	*trpC2* Δ*dgcW*::*cat*	GP715 → 168
GP983	*trpC2* Δ*cdaS*::*ermC*	*12*
GP997	*trpC2* Δ*cdaA*::*cat*	*12*
GP991	*trpC2* Δ*cdaS*::*ermC* Δ*disA*::*tet*	*12*
GP1341	*trpC2 ganA*::(p_{xyl} *cdaS aphA3*)	*12*
GP1381	*trpC2 cdaA*-FLAG *ermC*	*3*
GP2030	*trpC2* Δ*ktrD*::*tet*	GHB12 → 168
GP2048	*trpC2* Δ*ktrC*::*cat*	LFH → 168
GP2079	*trpC2* Δ*ktrC*::*tet*	LFH → 168
GP2083	*trpC2* Δ*ktrAB*::*aphA3* Δ*ktrC*::*tet*	GP2079 → GP92
GP2136	*trpC2* Δ*ktrAB*::*aphA3* Δ*ktrD*::*tet*	GP2030 → GP92
GP2165	*trpC2* Δ*ktrAB*::*aphA3* Δ*kimA*::*cat*	GP93 → GP92
GP2167	*trpC2* Δ*ktrD*::*tet* Δ*kimA*::*cat*	GP2030 → GP93
GP2169	*trpC2* Δ*ktrAB*::*aphA3* Δ*ktrC*::*cat* Δ*ktrD*::*tet*	GP2048 → GP2136
GP2182	*trpC2 amyE*::(p_{kimA}-*lacZ cat*)	pGP2915 → 168
GP2198	*trpC2 amyE*::(P_a-RS-*lacZ cat*)	pGP2916 → 168
GP2200	*trpC2 ganA*::(p_{xyl} *cdaS aphA3*) *amyE*::(p_a-RS-*lacZ cat*)	pGP2916 → GP1341
GP2209	*trpC2* Δ*cdaA*::*spc* Δ*cdaS*::*ermC*	GP94 → GP983
GP2222	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet*	GP997 → GP991

GP2223	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{S187F}$	Suppressor isolation of GP2222
GP2226	*trpC2* Δ*nhaK*::*aphA3*	LFH → 168
GP2227	*trpC2* Δ*cdaS*::*ermC* Δ*disA*::*tet* Δ*nhaK*::*aphA3*	GP2226 → GP991
GP2228	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* Δ*nhaK*::*aphA3*	GP997 → GP2227
GP2229	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{R22W}$	Suppressor isolation of GP2222
GP2230	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{P349L}$	Suppressor isolation of GP2222
GP2231	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{L391P}$	Suppressor isolation of GP2222
GP2232	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{F23L}$	Suppressor isolation of GP2222
GP2233	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{P553L}$	Suppressor isolation of GP2222
GP2234	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{T53I}$	Suppressor isolation of GP2222
GP2235	*trpC2* Δ*cdaA*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* *nhaK*$_{A395V}$	Suppressor isolation of GP2222
GP2236	*trpC2* Δ*dgcW*::*cat* Δ*cdaS*::*ermC* Δ*disA*::*tet* Δ*nhaK*::*aphA3*	GP850 → GP2227
GP2279	*trpC2 cdaA*-FLAG *ermC disA-Strep spc*	BP610 → GP1381
GP2405	*trpC2 kimA*-3×FLAG *spc*	pGP2789 → 168

Table S1. Bacterial strains used in this study. Arrows indicate construction by transformation. LFH, long flanking homology PCR.

Plasmid	Description	Construction/ Reference
pAC5	Vector for the construction of translational *lacZ* fusions that can be integrated at the *amyE* site.	*31*
pAC6	Vector for the construction of transcriptional *lacZ* fusions that can be integrated at the *amyE* site.	*32*
pAC7	Vector for the construction of translational *lacZ* fusions that can be integrated at the *amyE* site.	*33*
pBP135	pAC5/ EcoRI+BamHI	PCR prod. *kimA* native promoter + riboswitch FC274/275/ EcoRI/BamHI
pBP164	pAC6/ EcoRI/BamHI	$P_a$promoter LS27/LS28/ EcoRI/BamHI
pBP599	pGP1389/ BamHI/SalI	PCR prod. *disA* FC332/333 BamHI/SalI
pBP372	pWH844/ EcoRI/BamHI	PCR prod. *ktrAB*JH53/JH54/ EcoRI/BamHI
pGP1331	Integrative vector for the fusion of 3x FLAG tag to the C-terminus of a protein, keeping expression under the natural promotor.	*40*
pGP1389	Integrative plasmid for the fusion of the C-terminus of a protein to a Strep tag, keeping the control of expression under control of the natural promotor of the target gene.	*34*
pGP2789	pGP1331/ BamHI/SalI	PCR prod. *kimA* MW13/14/ BamHI/SalI
pGP2912	pAC6/ EcoRI+BamHI	PCR prod. *kimA* native promoter + riboswitch FC274/275/ EcoRI/BamHI
pGP2913	pWH844/BamHI+PstI	PCR prod. *kimA* JN465/466/ BamHI/PstI
pGP2915	pAC6/ EcoRI+BamHI	PCR prod. *kimA* native promoter - riboswitch FC274/JN493/ EcoRI/BamHI
pGP2916	pAC5/ EcoRI+BamHI	PCR prod. *kimA* artificial promoter4 + riboswitch JN570/FC275/ EcoRI/BamHI

pGP2917	pAC7/ EcoRI+BamHI	PCR prod. *kimA* artificial promoter4 + riboswitch JN570/FC275/ EcoRI/BamHI
pGP2920	pAC6/ EcoRI+BamHI	PCR prod. *kimA* artificial promoter4 + riboswitch JN570/FC275/ EcoRI/BamHI
pWH844	Fusion of a His-tag at the N-terminus of a protein for inducible overexpression via IPTG in *E. coli.*	*35*

LacZ fusion primers

FC274	AAAGAATTCGATTTTAGCCTCTGTTTTTTTATTTTTG GTAAGTAAATTCC	fwd_*kimA* riboswitch, EcoRI
FC275	TTTGGATCCATCGATGTCTTCCCCTTTTAATTTCTCA TTTTTCAATTAAAACAATAAAACATCC	rev_*kimA* riboswitch, BamHI
JN493	TTTGGATCCGCCGTTCTATTGGGTCCCC	rev_*kimA* promoter region (-riboswitch), BamHI
JN570	AAAGAATTC*TTGTCAAGTGAAGGCGCGCTATGCTA CAAT*CTCTTTTAGAAAACAAATCGCTTAATCTGAA	fwd_*kimA* riboswitch p_a, EcoRI
LS27	AATTCTTGTCAAGTGAAGGCGCGCTATGCTACAAT ACAGCTTGGAAATG	fwd_p_a
LS28	GATCCATTTCCAAGCTGTATTGTAGCATAGCGCGC CTTCACTGACAAG	rev_p_a

Primers used for the construction of deletion mutants

CD213	GCTTCCTGACAAATACAGAACAGTCATCG	LFH_*cdaA*_up_fwd
CD214	**CCTATCACCTCAAATGGTTCGCTG**GAATATCAACG GCATTGCCGAGGTAC	LFH_*cdaA*_up_rev, **kan^R**
CD219	GCCTGAAGATGCAGTTGTATCGCTG	LFH_*cdaA*_seq_fwd
CD230	**CCGAGCGCCTACGAGGAATTTGTATCG**CACCAGA GACACTTCTTCTAACCGC	LFH_*cdaA*_down_fwd, **kan^R**
CD231	CCGCTTCACTATCGACTTCTATATGC	LFH_*cdaA*_down_rev
CD232	GAGAGCCTTTAGCCGTTACATCTATC	LFH_*cdaA*_seq_rev
JN247	**CCTATCACCTCAAATGGTTCGCTG**CAAATACTGCC ACCAAAACGGC	LFH_*ktrC*_up_rev, **kan^R**
JN248	GTCTTTCAAGTGGTTTTGCCTTTAAAAT	LFH_*ktrC*_up_fwd

JN249	**CCGAGCGCCTACGAGGAATTTGTATCG**GTTACAGACATCTCCCGTTTCGA	LFH_*ktrC*_down_fwd, **kan^R**
JN250	GAAAAAGAACCGGCGTGCG	LFH_*ktrC*_down_rev
JN251	GAAAAGGGCACTGGACTGGG	LFH_*ktrC*_seq_fwd
JN252	CTTGAATCGCCATTTTCACCTGAT	LFH_*ktrC*_seq_rev
JN636	**CCTATCACCTCAAATGGTTCGCTG**CGTTAATAATACGAGCACAACTAAAAATATGT	LFH_*nhaK*_up_rev, **kan^R**
JN637	CGTATCATTTCGATCATAACGGCG	LFH_*nhaK*_up_fwd
JN638	**CCGAGCGCCTACGAGGAATTTGTATCG**CATTAATGATGTCGAAGCCGCC	LFH_*nhaK*_down_fwd, **kan^R**
JN639	CTCTTGCCACACCGGATTCT	LFH_*nhaK*_down_rev
JN640	CGTAAAAGAAGAATTGGAAAACCGTC	LFH_*nhaK*_seq_fwd
JN641	GTTTCATAGCCTTGATCTTCATTCCA	LFH_*nhaK*_seq_rev
KG301	GACGTTACAGCAAATGATGCGAAAGGACG	LFH_*kimA*_up_fwd
KG302	**CCTATCACCTCAAATGGTTCGCTG**GAATGATACATCGATGTCTTCCCCTTTTAATTTCTC	LFH_*kimA*_up_rev, **kan^R**
KG303	**CCGAGCGCCTACGAGGAATTTGTATC**CACACCATCCTTCACAACCAATCGGC	LFH_*kimA*_down_fwd, **kan^R**
KG304	GACTTCGCCAGAAGAAACCTGTCCTG	LFH_*kimA*_down_rev
KG305	CGTAAGCCAATCGAAGCTAGAGGAGC	LFH_*kimA*_seq_fwd
KG306	GGAAGTGATTCCGCGCTATGTAC	LFH_*kimA*_seq_rev

Primers used for the construction of *E. coli kimA* expression

JH53	AAAGAATTCAAGGGAGATATGAACATTGGGAAGAATTAAAAATAAG	fwd_*ktrAB*, native RBS, EcoRI
JH54	TTTGGATCCTCACCCTGTAAACACTTCGCCATCA	rev_*ktrAB,* BamHI
JN465	AAAGGATCCTGAGAAATTAAAAGGGGAAGACATCG	fwd_*kimA,* native RBS, BamHI
JN466	TTTCTGCAGTTACTTTTTAAAATGATACGGCAGTGTGGCAAC	rev_*kimA,* PstI

Primers used for KimA overexpression

MW13	AAAGGATCCGGCGCTCTGATATCATTTATGGTTC	fwd_*kimA,* BamHI
MW14	TTTGTCGACCTTTTTAAAATGATACGGCAGTGTGG	rev_*kimA,* SalI

Primers used for DisA FLAG-tag

FC332	AAAGGATCCCAAACACTTGAAAAATATAAGACAATC CTCGATAAAACG	fwd_d*isA,* BamHI
FC333	TTTGTCGACCAGTTGTCTGTCTAAATAATGCTTCTC TTGCAG	rev_*disA,* SalI

Internal primer pairs to *disA, cdaS cdaA, cdaR,* and *dgcW*

FX20	GGGGCGAAACACGAGTTAG	fwd_*disA*
FX21	GTCCACCACTTCTTTTACTTTATC	rev_*disA*
FX22	GCGTTCAAGGGGAAAATACAG	fwd_*cdaS*
FX23	GGATGCAATCCCCTGCATG	rev_*cdaS*
FX24	GATATATAAATTGATTATGGTGATACGC	fwd_*cdaA*
FX25	AATCCCCATGTTATCGCTTGGT	rev_*cdaA*
FX26	GGTTAACAGCAACCAAGCAC	fwd_*cdaR*
FX27	ACCGTTTGCGGAACACCC	rev_*cdaR*
FX38	GTCCATATGGAAATGCCGATG	fwd_*dgcW*
FX39	GCCAAGCACAACTGATCCG	rev_*dgcW*

Table S2. Plasmids and oligonucleotides used in this study.