Supporting Information

López *et al*. 10.1073/pnas.0810940106

1. Ivey DM, *et al.* (1993) Cloning and characterization of a putative Ca2+/H+ antiporter gene from Escherichia coli upon functional complementation of Na+/H+ antiporter-deficient strains by the overexpressed gene. *J Biol Chem* 268:11296–11303.
2. Reuber TL, Ausubel FM (1996) Isolation of Arabidopsis genes that differentiate between resistance responses mediated by the RPS2 and RPM1 disease resistance genes. *Plant Cell* 8:241–249.
3. Holtmann G, Bakker EP, Uozumi N, Bremer E (2003) Isolation of Arabidopsis genes that differentiate between resistance responses mediated by the RPS2 and RPM1 disease resistance genes. *J Bacteriol* 185:1289–1298.
4. Endo T, Uratani B, Freese E (1983) Purine salvage pathways of Bacillus subtilis and effect of guanine on growth of GMP reductase mutants. *J Bacteriol* 155:169–179.

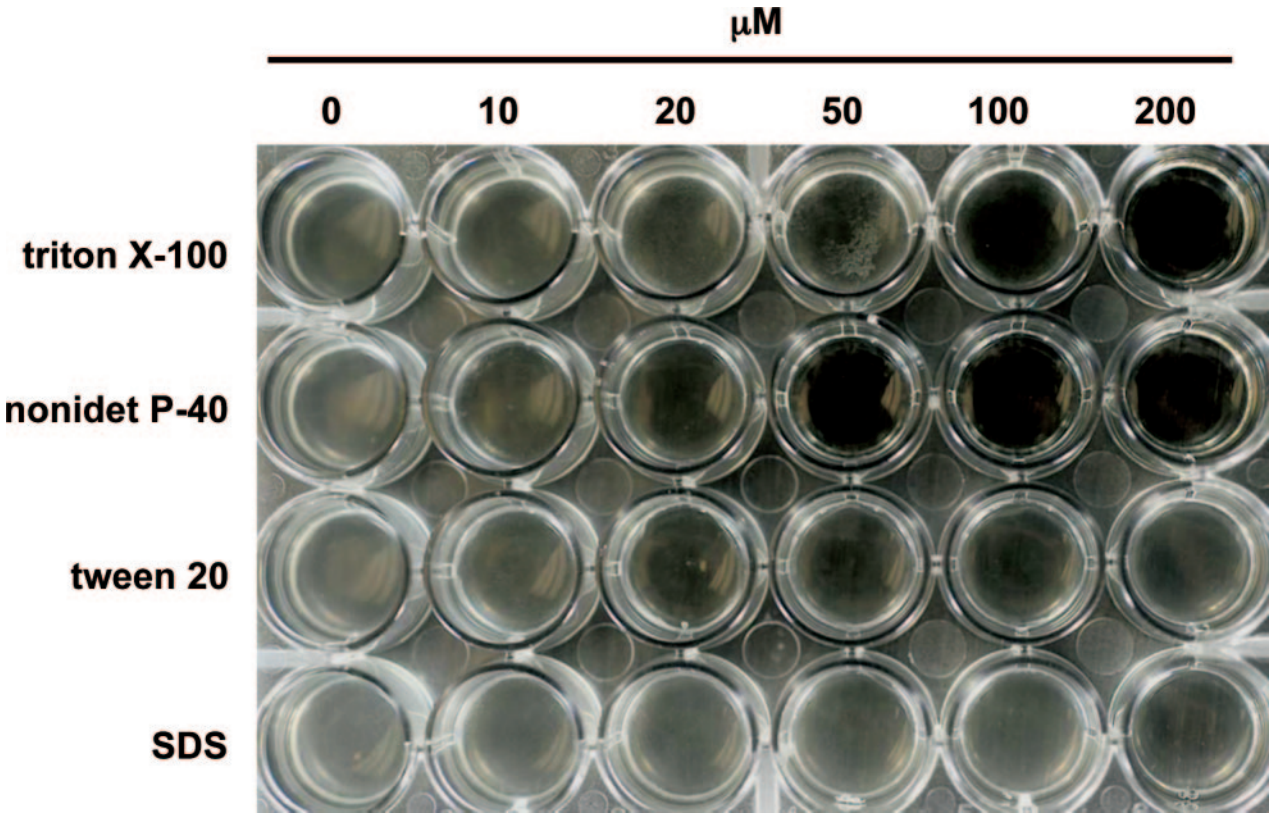

Fig. S1. Membrane disruption is not the mechanism associated to surfactin and nystatin in biofilm formation. Pellicle formation assays were performed to test biofilm formation effects by the addition of detergents. Detergents listed were tested in different concentrations to determine whether they induced pellicle formation. No pellicle formation ability was associated with any of them. Cell lysis was observed at concentrations above 50 μM.

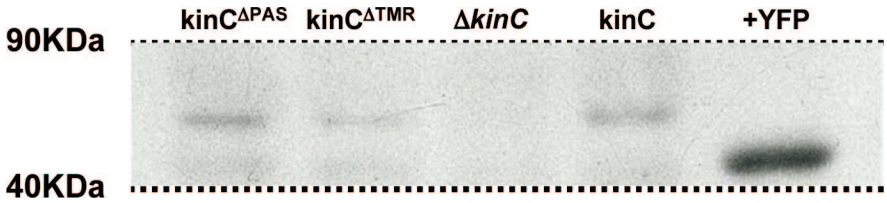

Fig. S2. Western immunoblot to confirm the expression of the KinC variants in *B. subtilis*. All constructs that contained a fused YFP could be detected with anti-YFP antibodies. Interestingly, the *kinC*$^{\Delta TMR}$ allele yields less protein, which might explain the reduced complementation seen in Fig. 3*C*. These results thus strongly argue that the PAS-PAC domain of KinC is responsible for sensing potassium leakage.

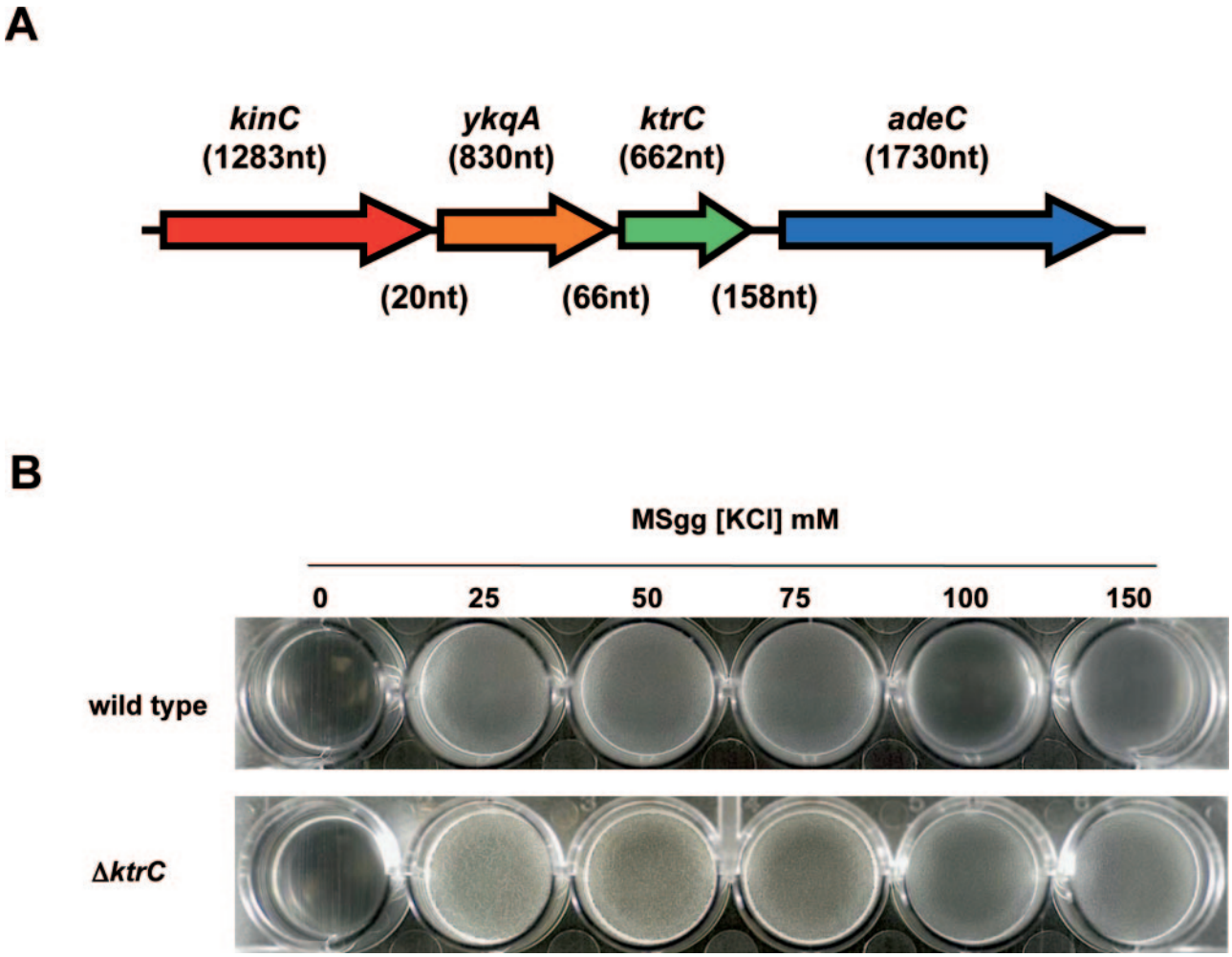

Fig. S3. (*A*) The *kinC* genetic locus. The 3 genes downstream of *kinC* are shown. The predicted proteins encoded by these 3 genes display sequence similarity to *ykqA*–AIG2 and ChaC family of proteins that bind small ligands. Both families have unknown function, but they might be related to the transport of cations (1, 2). *ktrC*, potassium channel regulator (3); adeC, adenine deaminase related to purine salvage (4). (*B*) Deletion of *ktrC* enhances biofilm formation possibly by affecting potassium reuptake. Wild type and Δ*ktrC* were grown in MSgg medium with different concentrations of potassium. In the absence of potassium, no growth was detected as expected because potassium is essential. At 25 mM potassium the Δ*ktrC* mutant forms a much more robust biofilm than the wild type. However, for both wild-type and mutant strains, biofilm formation is still suppressed by high concentrations of potassium.

Table S1. Small molecules/natural products tested

Molecule	Function	Structure	Pellicle formation
Nystatin	Cation-selective pore former	Macrolide polyene	Yes
Amphotericin	Cation-selective pore former	Macrolide polyene	Yes
Gramicidin	Cation-selective pore former	Peptide	Yes
Surfactin	Cation-selective pore former	Lipopeptide	Yes
Valinomycin	Potassium-selective ionophore	Peptide	Yes
Filipin	Cell wall disaggregator	Macrolide polyene	No
Iturin	Anion-selective pore former	Lipopeptide	No
Nisin	Nonselective pore former	Peptide	No
Polymyxin	Cell wall disaggregator	Lipopeptide	No
Bacitracin	Cell wall synthesis inhibitor	Peptide	No
Vancomycin	Cell wall synthesis inhibitor	Glycopeptide	No
Aculeacin	Cell wall synthesis inhibitor	Lipopeptide	No
Microcystin	Protein phosphatase inhibitor	Peptide	No
Syringomycin	Nonselective pore former	Lipopeptide	No
Nonactin	Ammonium-selective pore former	Peptide	No
Novobiocin	DNA gyrase inhibitor	Glycoside	No
Stigmatellin	Electron transport inhibitor	Quinone polyene	No
Antimycin	Electron transport inhibitor	Lipopeptide	No

Table shows a battery of small molecules tested for their ability to induce the formation of pellicle in LB cultures of *B. subtilis*. The molecules were selected according to their functionality or their similarly to surfactin or nystatin. Purified molecules and information about their mechanism of action were obtained from Sigma-Aldrich. Concentrations used covered a range between 0 and 100 μM. Growth inhibition and cell lysis were not observed at any concentration tested with the following natural products: nystatin, amphotericine, surfactin, iturin, aculeacin, and microcystin. For valinomycin and gramicidin, pellicle formation was observed at 3 μM, 10-fold less than the concentration needed for growth inhibition.

Table S2. Strain list

Strain	Genotype	Source
DL1	NCIB3610 wild type	1
DL5	Δ*sinR*::*spc*	2
DL107	Δ*srfAA*::*mls*	1
SSB602	Δ*sinR*::*spc* Δ*srfAA*::*mls*	This study
DL227	Δ*kinC*::*mls*	3
DL153	Δ*kinD*::*tet*	This study
DL99	Δ*kinC*::*mls* Δ*kinD*::*tet*	This study
DL340	Wild type *amyE*::*kinC*	This study
DL348	Wild type *amyE*::*kinC*$^{\Delta TMR}$	This study
DL356	Wild type *amyE*:: *kinC*$^{\Delta PAS}$	This study
DL344	Δ*kinC*::*mls amyE*::*kinC*	This study
DL352	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta TMR}$	This study
DL360	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta PAS}$	This study
DL368	Δ*kinD*::*tet amyE*::*kinC*	This study
DL370	Δ*kinD*::*tet amyE*::*kinC*$^{\Delta TMR}$	This study
DL372	Δ*kinD*::*tet amyE*::*kinC*$^{\Delta PAS}$	This study
DL374	Δ*kinC*::*mls* Δ*kinD*::*tet amyE*::*kinC*	This study
DL376	Δ*kinC*::*mls* Δ*kinD*::*tet amyE*::*kinC*$^{\Delta TMR}$	This study
DL378	Δ*kinC*::*mls* Δ*kinD*::*tet amyE*::*kinC*$^{\Delta PAS}$	This study
DL79	Wild type *amyE*::P$_{yqxM}$-*lacZ*	This study
DL382	Wild type *amyE*::P$_{yqxM}$-*yfp*	This study
DL215	Δ*kinC*::*mls amyE*::P$_{yqxM}$-*yfp*	This study
DL456	Δ*kinC*::*mls lacA*::P$_{yqxM}$-*yfp*	This study
DL449	Δ*kinC*::*mls amyE*::*kinC lacA*::P$_{yqxM}$-*yfp*	This study
DL451	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta TMR}$ *lacA*::P$_{yqxM}$-*yfp*	This study
DL453	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta PAS}$ *lacA*::P$_{yqxM}$-*yfp*	This study
DL346	Δ*kinC*::*mls amyE*::*kinC-yfp*	This study
DL354	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta TMR}$-*yfp*	This study
DL362	Δ*kinC*::*mls amyE*::*kinC*$^{\Delta PAS}$-*yfp*	This study
DL447	Δ*degS*::*tet*	This study
DL474	Δ*degS*::*tet amyE*::*kinC-degS*	This study
DL492	Δ*degS*::*tet amyE*::*kinC-degS lacA*::P$_{aprE}$-*lacZ*	This study
DL272	Δ*ktrC*::*tet*	This study
DL987	*L. monocytogenes tRNA*$^{Arg}\Omega$ pPL2-P$_{skf}$-*cfp*	This study
DL989	*L. monocytogenes tRNA*$^{Arg}\Omega$ pPL2-*kinC-spo0A*-P$_{skf}$-*cfp*	This study
DL1046	*L. monocytogenes tRNA*$^{Arg}\Omega$ pPL2 -*kinC*$^{\Delta PAS}$-*spo0A*-P$_{skf}$-*cfp*	This study

1. Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R, Kolter R (2001) Fruiting body formation by Bacillus subtilis. *Proc Natl Acad Sci USA* 98(20):11621–11626.
2. Heytler PG, Prichard WW (1962) A new class of uncoupling agents—Carbonyl cyanide phenylhydrazones. *Biochem Biophys Res Commun* 7:272–275.
3. Magnuson R, Solomon J, Grossman AD (1994) Biochemical and genetic characterization of a competence pheromone from B. subtilis. *Cell* 77(2):207–216.

Table S3. Primer list

Purpose	Name	Sequence (5′–3′)
Δ*degS*::*tet*	degS-1	agccctacaactaccaatagtgcaa
	degS-2	caattcgccctatagtgagtcgtagcactttggaatccatctttgtttt
	degS-3	ccagcttttgttccctttagtgagataggtcttgggacatttattatgatt
	degS-4	tcaatttcttcacggtaagtctcct
Δ*kinD*::*tet*	kind-1	tcttcttgtgattaacccgccaaga
	kind-2	gaacaacctgcaccattgcaagaagtattttcaatttgcatcgctccaa
	kind-3	ttgatccttttttttataacaggaattcttcatattgaaagtgaagtgcgaaga
	kind-4	tgtttaagatattcttcacctgggta
Δ*ktrC*::*tet*	krtC-1	gaggaacaacgaaggcg
	ktrC-2	gagaacaacctgccattgcaagatgctttgcaaatactgc
	ktrC-3	gggatcaactttgggagagagttcgcaaccgaagtcatccac
	ktrC-4	cgaactcaatcggtgtaac
Transcriptional fusions	PyqxMfw	tggcgaattctcagagttaaatggtattgcttcact
	PyqxMrv	cctaagcttgtaaaacactgtaacttgatatgacaa
	PaprEfw	aaaagaattctcttcctccctctcaata
	PapreErv	ttttggatcccgcaaacaacaagctgatc
kinC alleles	KcPASupfw	aaaagaattcgtcatgccgattgagttgag
	KcPASuprv	ctgggattccctcgccagttcagaaagctgtttatacttc
	KcPASdwfw	ctggcgagggaatcccag
	KcPASdwrv	ttttggatccgtatacaaacagaagcgag
	KcTMRuprv	accagctgctgtttctcatcccattgatattttctcatatgaccacc
	KcTMRdwfw	gatgagaaacagcagctggt
Chimera	KinCCHIupfw	aaaaaagcttgtcatgccgattgagttgag
	KinCCHIuprv	tgatagtttctgggattcc
	Degsupfw	ggaatcccagaaactatcagagcgaaaaagagtctcaa
	Degsdwrv	ttttggatccccgtcatcaccttcggc
System expressed in *L. monocytogenes*	Kinc-up	cactcttcattacgctgcttggccgcattgtcatgcc
	kinC-dw	gatcgagcttatgtatacac
	Spo0A-up	gtgtatacataagctcgatcgaaaattaccgatccaagac
	Spo0a-dw	aaaagtcgactacttacgattttgcaggac
	Pskf-up	aagcagcgtaatgaagagtc
	Pskf-upSalI	aaaagtcgacaagcagcgtaatgaagagtg
	Pskf-cfp-dw	aaaacggccgtgatcgaaatagtacataatg