Supporting Information

ABC transporters required for export of wall teichoic acids do not discriminate between different main chain polymers

Kathrin Schirner, Laura K. Stone and Suzanne Walker*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA

*Corresponding author: suzanne_walker@hms.harvard.edu

Table S1. Strains, plasmids and oligonucleotides.

strain	genotype	reference
B. subtilis		
PY79	Prototrophic derivative of *B. subtilis* 168	(*1*)
KS001	PY79 *amyE::*$P_{hyperspank}$ *tarGH*^{Sa} *spc*	this work
KS002	PY79 Δ*tagGH*^{Bs}*::cat amyE::*$P_{hyperspank}$ *tarGH*^{Sa} *spc*	this work
KS003	PY79 *tagH* Δaa276-527	this work
KS004	PY79 *amyE::*$P_{hyperspank}$ *tagGH*^{Bs} *spc*	this work
KS005	PY79 Δ*tagGH*^{Bs}*::cat amyE::*$P_{hyperspank}$ *tagGH*^{Bs} *spc*	this work
E. coli		
XL1blue	*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17(r_K-, m_K+) *sup*E44 *rel*A1 *lac* [F', *proAB*, *lac*IqZΔM15::Tn10(tetr)]	Promega
plasmid	**genotype**	**reference**
pDR111	*amyE::*$P_{hyperspank}$ *spc*	D. Rudner
pKM074	*bla cat*	D. Rudner
pMAD	*ori*^{BsTs} *lacZ amp mls*	(*2*)
pKS001	pDR111 *tarGH*^{Sa}	this work
pKS002	pDR111 *tarGH*^{Bs}	this work

primer	sequence	features / use
1	catggtcgacacataaggaggaactactaTGAAAGTGTGGTTTAATG	RBS; SalI; amplification of *tarG*^{Sa}
2	ctgagtctagaTTACAAGAAGTCTGCAAATTG	XbaI; amplification of *tarG*^{Sa}
3	cagtctagaacataaggaggaactactATGAACGTTTCGGTAAAC	RBS, XbaI; amplification of *tarH*^{Sa}
4	ctagtcgcatgcTTATTTAATAACGAAGCG	SphI; amplification of *tarH*^{Sa}
5	gactatctagaCTTTACACGGGCGTTTC	XbaI; up-stream region of *tagG*, for deletion of *tagGH*^{Bs}
6	agtcaggatccGTATACGCAACAAATCATTC	BamHI; up-stream region of *tagG*, for deletion of *tagGH*^{Bs}
7	acagtcggccgCATACACTGACTTTGATGTTG	EagI; down-stream region of *tagH*, for deletion of *tagGH*^{Bs}
8	ctgaggtcgacCGTGAAGGAATTGTAATACG	SalI; down-stream region of *tagH*, for deletion of *tagGH*^{Bs}
9	CTTACAggatccGGTTGTTCACATTCCTTTTATTG	BamHI; up-stream region including 5' end of *tarH*^{Bs} for truncation
10	GATGAtctagaTTATTTCTTCTTTTTGCGGAAACG	XbaI; upstream region including 5' end of *tarH*^{Bs}; stop codon for truncation after aa 275
11	CGATGtctagaTAAAAAAAGGCTATTGGATGAATG	XbaI; downstream region of *tarH*^{Bs}
12	GTAGTgaattcGAAGTCTTTATCAGGCTAAGG	EcoRI; downstream region of *tarH*^{Bs}
13	catgaagcttacataaggaggaactactaGTCTAAGGAAGATAAAAAATG	HindIII; amplification of *tagGH* from *B. subtilis*
14	gctagtcgctagcGACATTCATCCAATAGCC	NheI; amplification of *tagGH* from *B. subtilis*

Table S2. Minimal inhibitory concentrations of various antibiotics against strains PY79, KS001 and KS002.

	Targocil	Kanamycin	Carbenicillin	Tunicamycin	PenicillinG	Erythromycin
wt	> 200 µM	0.2-0.3 µg/ml	0.16 µg/ml	0.125 µg/ml	0.03 µg/ml	0.06 µg/ml
KS001	> 200 µM	0.2-0.3 µg/ml	0.16 µg/ml	0.125 µg/ml	0.03 µg/ml	0.06 µg/ml
KS002	1.25 µM	0.2-0.3 µg/ml	0.16 µg/ml	0.125 µg/ml	0.03 µg/ml	0.06 µg/ml

Methods

Growth curves

The growth curve was obtained by diluting cultures into LB with or without 1 mM IPTG in a 96-well microtiter plate incubated at 37°C whilst shaking and taking OD_{600} measurements every 10 min or 30 min in a GloMax Multi Detection System (Promega).

Construction of a complementation strain

The genes *tagGH* from *B. subtilis* were amplified using primers 13+14 and the PCR product was cloned into pDR111 giving pKS002. The plasmid was transformed into *B. subtilis*, where it integrated into the *amyE* locus, giving strain KS004.

Then *tagGH* was deleted from KS004 as described for constructing KS002. Deletion of $tagGH^{Bs}$ was confirmed by PCR.

Transformation of *B. subtilis* was done according to the method of Anagnostopoulos and Spizizen (*23*) as modified by Jenkinson (*24*).

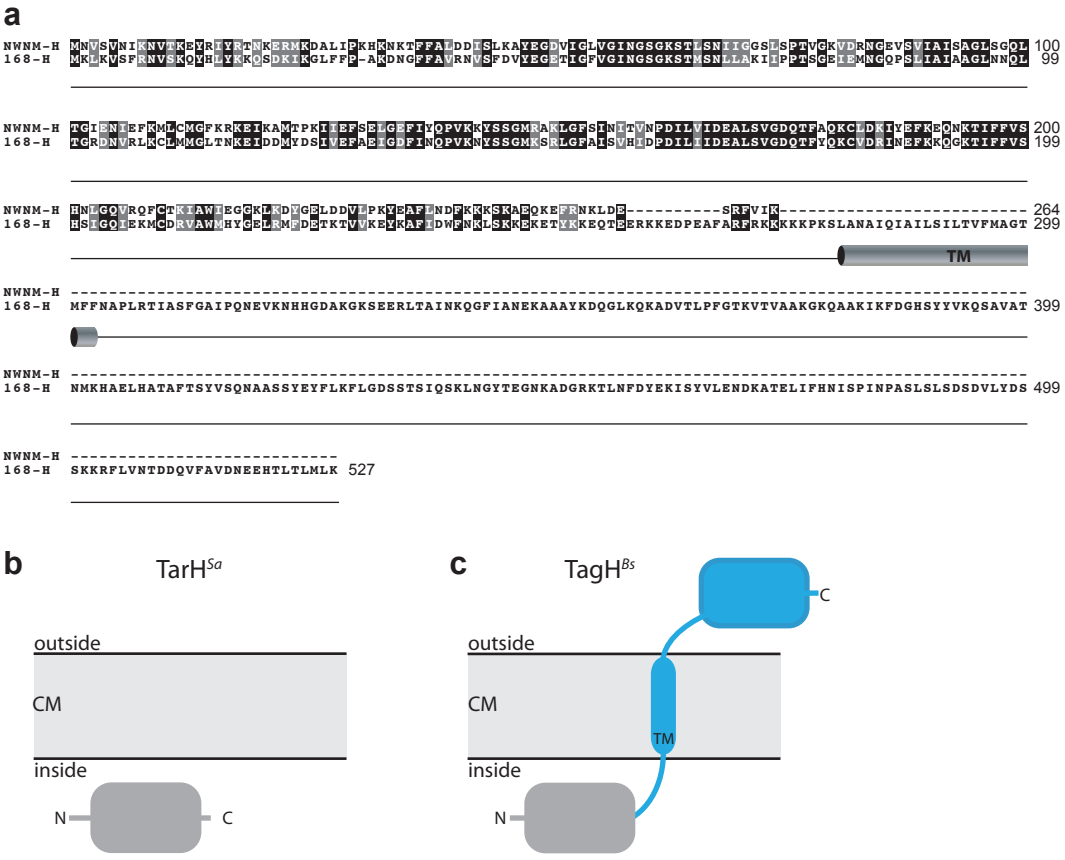
Construction of a TagH truncation mutant in *B. subtilis*

To construct *B. subtilis* PY79 with a truncated TagH, approximately 1000 bp up-stream of *tagH* was amplified using primers 9+10, including the 5' end of *tagH* until bp 525 (encoding for K275) after which a stop codon was inserted. A fragment of the downstream region starting with the stop codon of *tagH* was amplified using primers 11+12. Both fragments were ligated together, used as a PCR template and re-amplified using primer pair 9+12. This fragment was the cloned into pMAD (*2*). *B. subtilis* was transformed with the resulting plasmid selecting for erythromycin resistance on plates containing 200 µg/ml chromo-chloro-indolyl-galactopyranoside (X-gal) at 30°C. A single blue colony was picked and inoculated in LB containing erythromycin, grown for 3 h at 30°C and then shifted to 43°C. After 6 h, dilutions were plated on plates containing erythromycin and X-gal. A single blue colony was picked, inoculated in LB and grown over night at 30°C. Dilutions were plated on X-gal plates and white colonies were patched on plates containing either erythromycin or on X-gal. Erythromycin-sensitive white colonies were selected, and truncation of *tagH* was confirmed by PCR and sequencing.

References

1. Youngman, P., Perkins, J. B., and Losick, R. (1984) Construction of a cloning site near one end of *Tn*917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene, *Plasmid 12*, 1-9.
2. Arnaud, M., Chastanet, A., and Debarbouille, M. (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, Gram-positive bacteria, *Appl Environ Microbiol 70*, 6887-6891.

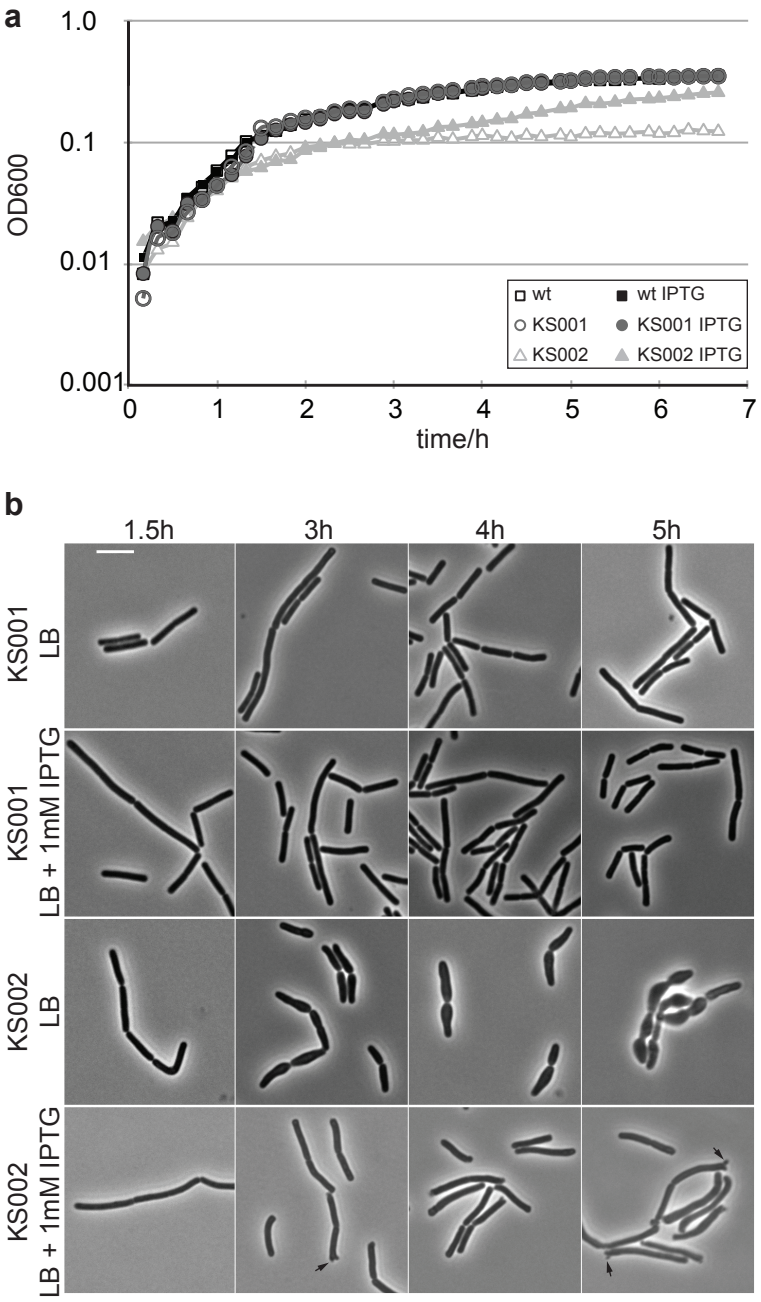
Figure S1



Supporting Figure S1. The ATPase component is partially conserved between *S. aureus* and *B. subtilis*

a. Alignment of TarH^{Sa} (NWMN-H) and TagH^{Bs} (168-H). The barrel indicates a predicted transmembrane helix. b. Topology prediction of TarH from *S. aureus*. c. Topology prediction of TagH from *B. subtilis* PY79.

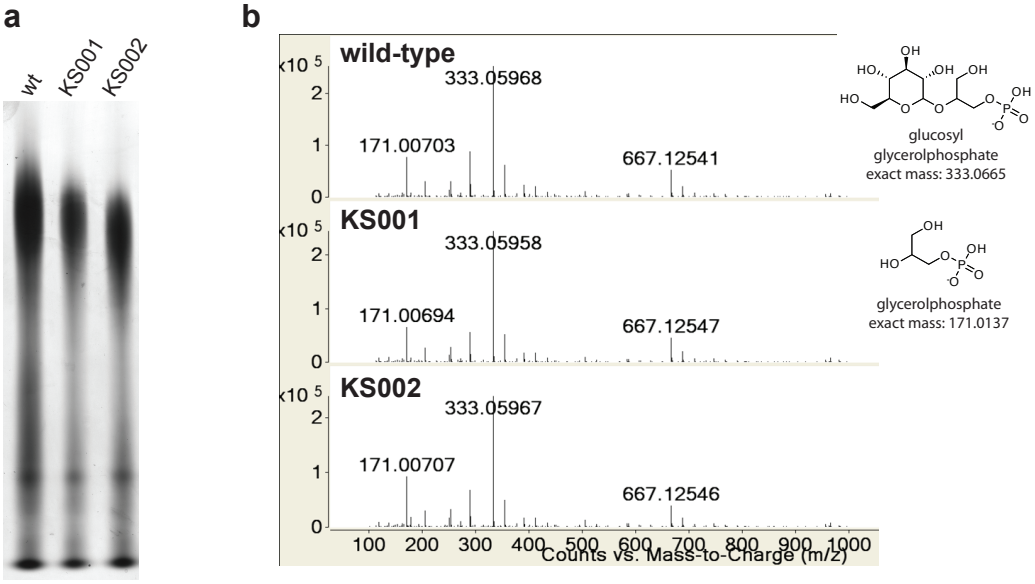
Figure S2



Supporting Figure S2. KS002 is dependent on the presence of IPTG in the medium.

a. Growth curve of strains PY79 (□), KS001 (○) and KS002 (△) in LB in the presence (closed symbols) and after washing away IPTG (open symbols). b. Time course microscopy of TarGH^{Sa} expression and depletion in KS001 and KS002. Arrows indicate malformed cell poles in KS003. Samples were taken from conditions and at the time points indicated. Scale bar 5 µm.

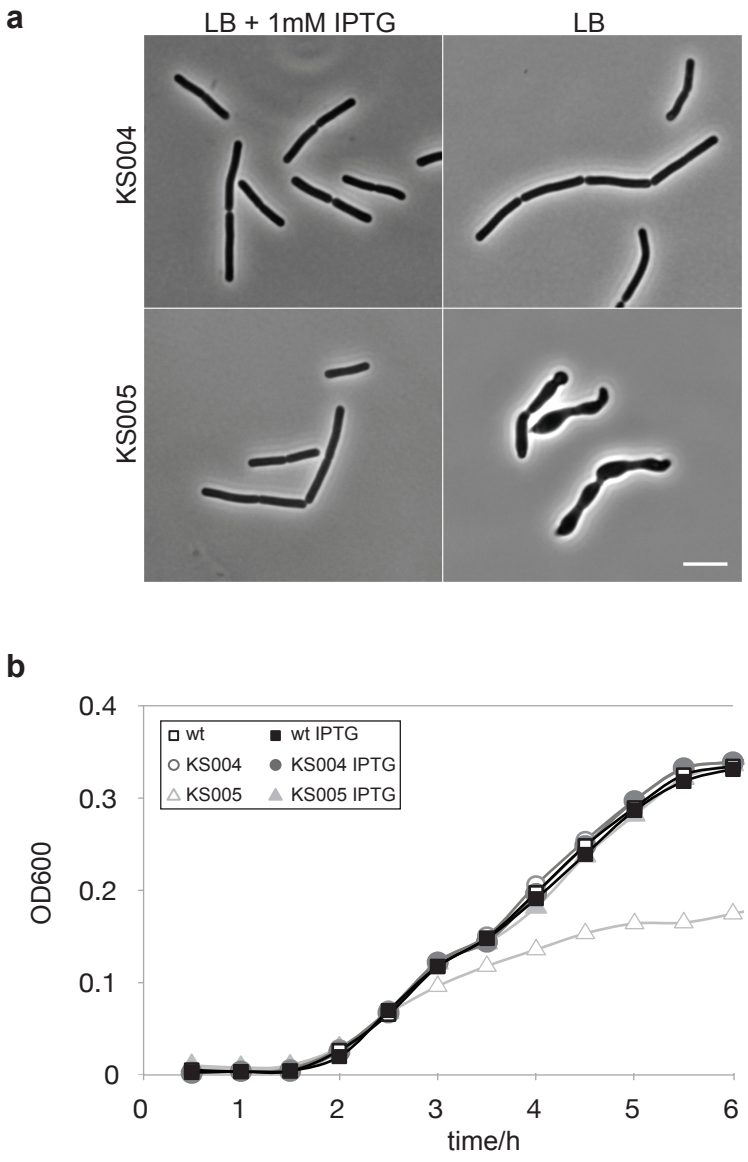
Figure S3



Supporting Figure S3. The WTA of wild-type, KS001 and KS002 is indistinguishable.

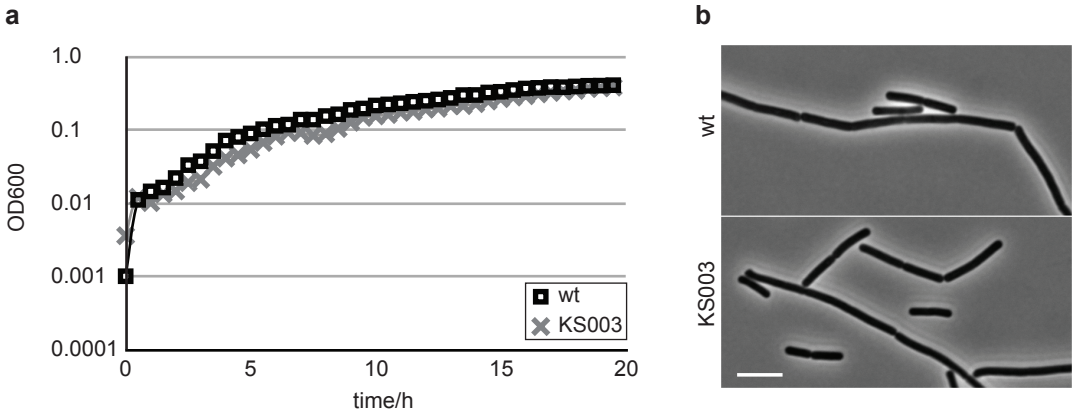
a. Gel showing the WTA pattern of *B. subtilis* PY79, KS001 and KS002. b. LC/MS data confirming the identity of the WTA monomers as glucosylated glycerol phosphates.

Figure S4



Supporting Figure S4. The endogenous WTA transporter TagGH^{Bs} complements the *tagGH* deletion.
a. Morphology of strains KS004 and KS005 grown in LB in the presence and absence of 1 mM IPTG. Scale bar 5 µm. b. Growth curve of wild-type, KS004 and KS005 in LB in the presence and after washing out IPTG.

Figure S5



Supporting Figure S5. Truncation of TarH has no effect on cell growth and morphology of *B. subtilis*.
a. Growth curve of wild-type (□) and strain KS003 (×)with a truncation of TagH. b. Phase contrast microscopic images of wild-type and KS003. Scale bar 5 μm.