Supporting Information for

Bacillus subtilis YngB contributes to wall teichoic acid glucosylation and glycolipid formation during anaerobic growth

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Table S1. Observed and predicted masses of parental and MS/MS fragments for the glycolipids (32:0) [DAG-Glc_2 + Na^+] and (32:0) [DAG-Glc_3 + Na^+].

Observed mass	Predicted mass	Chemical formula
(32:0) [DAG-Glc_2 + Na^+]		
915.6	915.6	(32:0) DAG-Glc-Glc + Na^+
673.5	673.3	(17:0) MAG-Glc-Glc – H_2O + Na^+
645.5	645.3	(15:0) MAG-Glc-Glc –H_2O + Na^+
405.2	405.1	$\text{CH}_2\text{=CH-CH}_2$-Glc-Glc + Na^+
365.1	365.1	Glc-Glc + Na^+
347.2	347.1	Glc-Glc – H_2O + Na^+
327.3	327.3	(17:0) MAG – OH
299.3	299.3	(15:0) MAG – OH
(32:0) [DAG-Glc_3 + Na^+]		
1077.8	1077.7	(32:0) DAG-Glc-Glc-Glc + Na^+
835.4	835.4	(17:0) MAG-Glc-Glc-Glc – H_2O + Na^+
807.4	807.4	(15:0) MAG-Glc-Glc-Glc – H_2O + Na^+
567.2	567.2	$\text{CH}_2\text{=CH-CH}_2$-Glc-Glc-Glc + Na^+
527.2	527.2	Glc-Glc-Glc + Na^+
509.1	509.2	Glc-Glc-Glc – H_2O + Na^+

MAG – monoacylgycerol; DAG – diacylglycerol; Glc – glucose. Note that only the observed masses for the glycolipids isolated from the wild-type strain are listed.

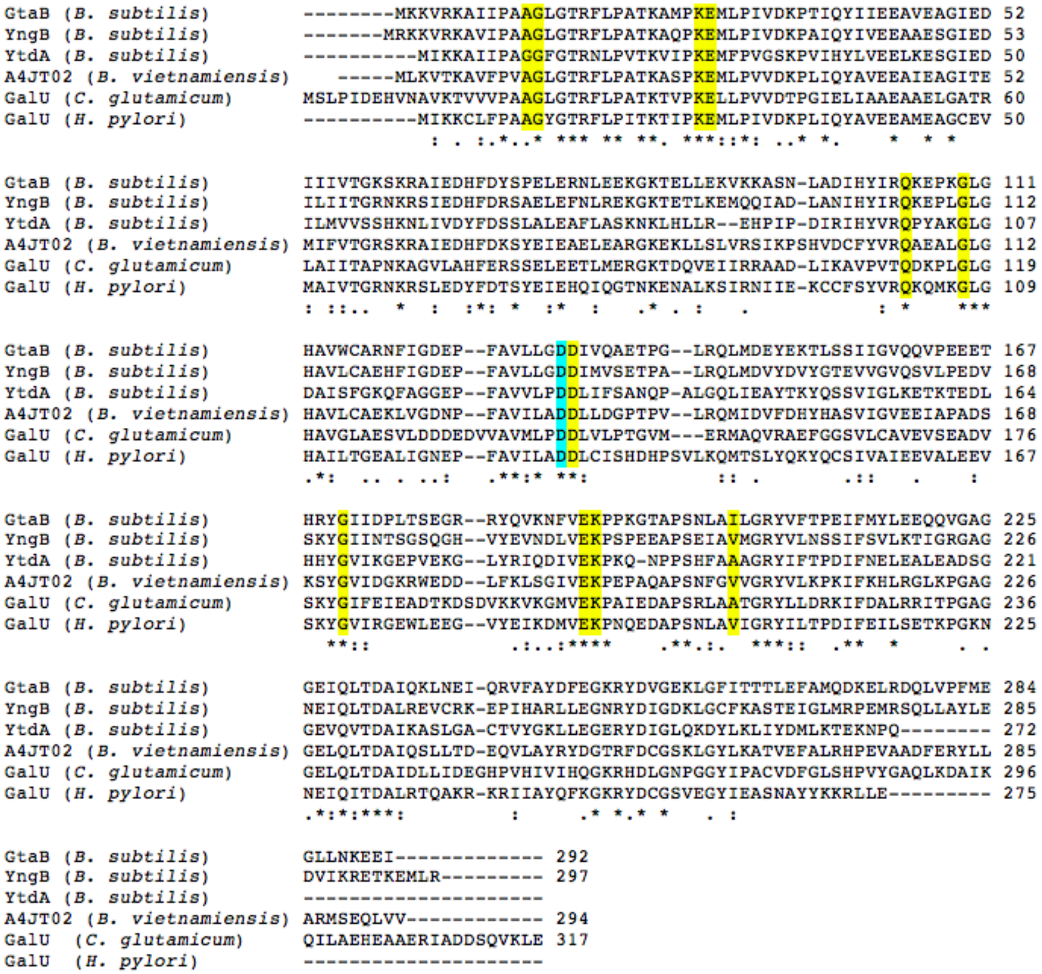
Table S2. Bacterial strains used in this study

Unique ID	Strain name and resistance	Source
***Escherichia coli* strains**		
ANG127	XL1-Blue	Stratagene
ANG191	BL21(DE3)	(1)
ANG5206	XL1-Blue pET28b-*gtaB*-cHis; KanR	This study
ANG5207	XL1-Blue pET28b-*yngB*-cHis; KanR	This study
ANG5208	BL21(DE3) pET28b-*gtaB*-cHis; KanR	This study
ANG5209	BL21(DE3) pET28b-*yngB*-cHis; KanR	This study
ANG680	DH5α pDR111; AmpR	(2)
***Bacillus subtilis* strains**		
ANG1691	168, trpC2	(3)
ANG5277	168Δ*gtaB::kan*; KanR	(4)
ANG5263	168Δ*yngB::kan*; KanR	(4)
ANG5658	168Δ*gtaB::erm*; ErmR	(4)
ANG5659	168Δ*yngB::erm*; ErmR	(4)
ANG5675	168, *amyE::spec* P_{hyper}; SpecR (**Short: WT P_{hyper}**)	This study
ANG5676	168Δ*gtaB::kan amyE::spec* P_{hyper}; KanR SpecR (**Short: Δ*gtaB* P_{hyper}**)	This study
ANG5677	168Δ*gtaB::kan amyE::spec* P_{hyper}-*gtaB*; KanR SpecR (**Short: Δ*gtaB* P_{hyper}-*gtaB***)	This study
ANG5678	168Δ*gtaB::kan amyE::spec* P_{hyper}-*yngB*; KanR SpecR (**Short: Δ*gtaB* P_{hyper}-*yngB***)	This study
ANG5679	168Δ*yngB::kan amyE::spec* P_{hyper}; KanR SpecR (**Short: Δ*yngB* P_{hyper}**)	This study
ANG5680	168Δ*yngB::kan amyE::spec* P_{hyper}-*yngB*; KanR SpecR (**Short: Δ*yngB* P_{hyper}-*yngB***)	This study
ANG5681	168Δ*gtaB::erm* Δ*yngB::kan amyE::spec* P_{hyper}; ErmR KanR SpecR (**Short: Δ*gtaB*/Δ*yngB* P_{hyper}**)	This study
ANG5682	168Δ*gtaB::kan* Δ*yngB::erm amyE::spec* P_{hyper}-*gtaB*; ErmR KanR SpecR (**Short: Δ*gtaB*/Δ*yngB* P_{hyper}-*gtaB***)	This study
ANG5683	168Δ*gtaB::erm* Δ*yngB::kan amyE::spec* P_{hyper}-*yngB*; ErmR KanR SpecR (**Short: Δ*gtaB*/Δ*yngB* P_{hyper}-*yngB***)	This study

Table S3. Primers used in this study

Number	Name	Sequence
ANG3161	5-NcoI-pET28b-*gtaB*-cHis	CATGCCATGGGCAAAAAAGTACGTAAAGCCATAATTCCAG
ANG3162	3-XhoI-pET28b-*gtaB*-cHis	CCGCTCGAGGCCGCTGCTGCCGCGCGGCACCAGGATTTCTTCTTTGTTTAGTAAACCTTC C
ANG3163	5-NcoI-pET28b-*yngB*-cHis	CATGCCATGGGCAGAAAAAAAGTGAGAAAAGCGGTTATAC
ANG3164	3-XhoI-pET28b-*yngB*-cHis	CCGCTCGAGGCCGCTGCTGCCGCGCGGCACCAGCCGCAGCATTTCTTTCGTTTCCCGTTTG
ANG111	5-pET28b-T7 promoter	TAATACGACTCACTATAGGG
ANG112	3-pET28b-T7 terminator	GCTAGTTATTGCTCAGCGG
ANG3197	5-*gtaB::kan*	TCATTTTAAATCATTTCATTCTTGATTC
ANG3198	3-*gtaB::kan*	ATTTATGTTTTCATTTAGTTTGTTTAAC
ANG3199	5-*yngB::kan*	TATTGCCGCGTCAGGGATCACCTT TTTG
ANG3200	3-*yngB::kan*	CTAATACAATCTCACTCGGAATGATTTC
ANG3203	5-HindIII-pDR111-*gtaB*	CCCAAGCTTAAAATAAGGAGGACCTTTTAAATGAAAAAAGTACGTAAAGC
ANG3204	3-NheI-pDR111-*gtaB*	CTAGCTAGCTTAGATTTCTTCTTTGTTTAGTAAACCTTC
ANG3205	5-HindIII-pDR111-*yngB*	CCCAAGCTTAAAATAAGGAGGAGAAGATGAATGAGAAAAAAAGTGAGAAAAG
ANG3206	3-NheI-pDR111-*yngB*	CTAGCTAGCTTACCGCAGCATTTCTTTCGTTTCCCGTTTG
ANG1663	5-pDR111-*amyE*-check	GCGAGGGAAGCGTTCACAGTTTCGGGC
ANG1664	3-pDR111-*amyE*-check	CGGTTGTAGCCCAAACGCCTTTCCGTGG
ANG1671	5-pDR111-seq	CGCTCTCCTGAGTAGGACAAATCCGC
ANG1672	3-pDR111-seq	CGGGAAACGGTCTGATAAGAGACACC

Supplemental Figures



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GtaB (B. subtilis)         --------MKKVRKAIIPAAGLGTRFLPATKAMPKEMLPIVDKPTIQYIIEEAVEAGIED 52
YngB (B. subtilis)         -------MRKKVRKAVIPAAGLGTRFLPATKAQPKEMLPIVDKPAIQYIVEEAAESGIED 53
YtdA (B. subtilis)         ----------MIKKAIIPAGGFGTRNLPVTKVIPKEMFPVGSKPVIHYLVEELKESGIED 50
A4JT02 (B. vietnamiensis)     -----MLKVTKAVFPVAGLGTRFLPATKASPKEMLPVVDKPLIQYAVEEAIEAGITE 52
GalU (C. glutamicum)       MSLPIDEHVNAVKTVVVPAAGLGTRFLPATKTVPKELLPVVDTPGIELIAAEAAELGATR 60
GalU (H. pylori)           ----------MIKKCLFPAAGYGTRFLPITKTIPKEMLPIVDKPLIQYAVEEAMEAGCEV 50
                                      : . :.*..* *** ** **. ***::*: ..* *.    *  * *

GtaB (B. subtilis)         IIIVTGKSKRAIEDHFDYSPELERNLEEKGKTELLEKVKKASN-LADIHYIRQKEPKGLG 111
YngB (B. subtilis)         ILIITGRNKRSIEDHFDRSAELEFNLREKGKTETLKEMQQIAD-LANIHYIRQKEPLGLG 112
YtdA (B. subtilis)         ILMVVSSHKNLIVDYFDSSLALEAFLASKNKLHLLR--EHPIP-DIRIHYVRQPYAKGLG 107
A4JT02 (B. vietnamiensis)  MIFVTGRSKRAIEDHFDKSYEIEAELEARGKEKLLSLVRSIKPSHVDCFYVRQAEALGLG 112
GalU (C. glutamicum)       LAIITAPNKAGVLAHFERSSELEETLMERGKTDQVEIIRRAAD-LIKAVPVTQDKPLGLG 119
GalU (H. pylori)           MAIVTGRNKRSLEDYFDTSYEIEHQIQGTNKENALKSIRNIIE-KCCFSYVRQKQMKGLG 109
                           : ::..  *  :  :*: *  :*  :   .* . :    .           : *    ***

GtaB (B. subtilis)         HAVWCARNFIGDEP--FAVLLGDDIVQAETPG--LRQLMDEYEKTLSSIIGVQQVPEEET 167
YngB (B. subtilis)         HAVLCAEHFIGDEP--FAVLLGDDIMVSETPA--LRQLMDVYDVYGTEVVGVQSVLPEDV 168
YtdA (B. subtilis)         DAISFGKQFAGGEP--FAVVLPDDLIFSANQP-ALGQLIEAYTKYQSSVIGLKETKTEDL 164
A4JT02 (B. vietnamiensis)  HAVLCAEKLVGDNP--FAVILADDLLDGPTPV--LRQMIDVFDHYHASVIGVEEIAPADS 168
GalU (C. glutamicum)       HAVGLAESVLDDDEDVVAVMLPDDLVLPTGVM---ERMAQVRAEFGGSVLCAVEVSEADV 176
GalU (H. pylori)           HAILTGEALIGNEP--FAVILADDLCISHDHPSVLKQMTSLYQKYQCSIVAIEEVALEEV 167
                           .*:  .. . ..:   .**:* **:           :: .       .::   .    :

GtaB (B. subtilis)         HRYGIIDPLTSEGR--RYQVKNFVEKPPKGTAPSNLAILGRYVFTPEIFMYLEEQQVGAG 225
YngB (B. subtilis)         SKYGIINTSGSQGH--VYEVNDLVEKPSPEEAPSEIAVMGRYVLNSSIFSVLKTIGRGAG 226
YtdA (B. subtilis)         HHYGVIKGEPVEKG--LYRIQDIVEKPKQ-NPPSHFAAAGRYIFTPDIFNELEALEADSG 221
A4JT02 (B. vietnamiensis)  KSYGVIDGKRWEDD--LFKLSGIVEKPEPAQAPSNFGVVGRYVLKPKIFKHLRGLKPGAG 226
GalU (C. glutamicum)       SKYGIFEIEADTKDSDVKKVKGMVEKPAIEDAPSRLAATGRYLLDRKIFDALRRITPGAG 236
GalU (H. pylori)           SKYGVIRGEWLEEG--VYEIKDMVEKPNQEDAPSNLAVIGRYILTPDIFEILSETKPGKN 225
                             **::            .:..:****    .**.:.  ***::  .**  *     . .

GtaB (B. subtilis)         GEIQLTDAIQKLNEI-QRVFAYDFEGKRYDVGEKLGFITTTLEFAMQDKELRDQLVPFME 284
YngB (B. subtilis)         NEIQLTDALREVCRK-EPIHARLLEGNRYDIGDKLGCFKASTEIGLMRPEMRSQLLAYLE 285
YtdA (B. subtilis)         GEVQVTDAIKASLGA-CTVYGKLLEGERYDIGLQKDYLKLIYDMLKTEKNPQ-------- 272
A4JT02 (B. vietnamiensis)  GELQLTDAIQSLLTD-EQVLAYRYDGTRFDCGSKLGYLKATVEFALRHPEVAADFERYLL 285
GalU (C. glutamicum)       GELQLTDAIDLLIDEGHPVHIVIHQGKRHDLGNPGGYIPACVDFGLSHPVYGAQLKDAIK 296
GalU (H. pylori)           NEIQITDALRTQAKR-KRIIAYQFKGKRYDCGSVEGYIEASNAYYKKRLLE--------- 275
                           .*:*:***:         :     .* *.* *   . :

GtaB (B. subtilis)         GLLNKEEI------------- 292
YngB (B. subtilis)         DVIKRETKEMLR--------- 297
YtdA (B. subtilis)         ---------------------
A4JT02 (B. vietnamiensis)  ARMSEQLVV------------ 294
GalU  (C. glutamicum)      QILAEHEAAERIADDSQVKLE 317
GalU  (H. pylori)          ---------------------
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Figure S1. Amino acid sequence alignment of *B. subtilis* UGPases and UGPases with available UDP-glucose bound structures. The amino acid sequences of the *Bacillus subtilis* 168 confirmed and predicted UGPases GtaB, YngB and YtdA were aligned with the protein sequence of A4JT02 from *Bulkholderia vietnamiensis,* GalU from *Corynebacterium glutamicum* and GalU from *Helicobacter pylori,* for which UDP-glucose bound structures are available using CLUSTALW (5). Based on the alignment and the structural information, possible UDP-glucose binding residues were identified and highlighted in yellow and a conserved aspartic acid likely involved in metal ion binding is highlighted in cyan.

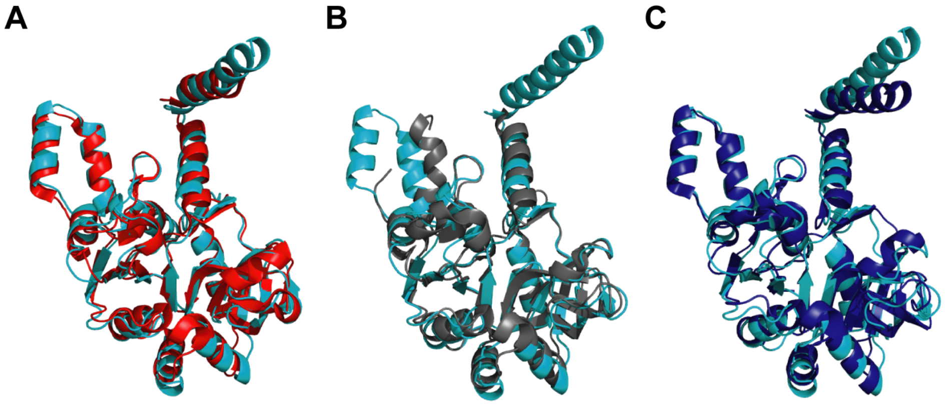

Figure S2. Superimposition of the *B. subtilis* YngB structure with structures of other UGPases. *A,* Superimposition of the *B. subtilis* YngB structure (cyan in all panels) with A4JT02 (PDB code: 5i1f) from *Bulkholderia vietnamiensis* (red) yielding an rmsd value of 1.39Å over 274 aligned residues*;* *B*, superimposition with GalU$_{\mathrm{Hp}}$ (PDB code: 3juk) from *Helicobacter pylori* (grey) giving an rmsd value of 1.61 Å over 250 aligned residues; and *C*, superimposition with GalU$_{\mathrm{Cg}}$ (PDB code 2pa4) from *Corynebacterium glutamicum* (blue) yielding an rmsd value of 2.11 Å over 282 aligned residues.

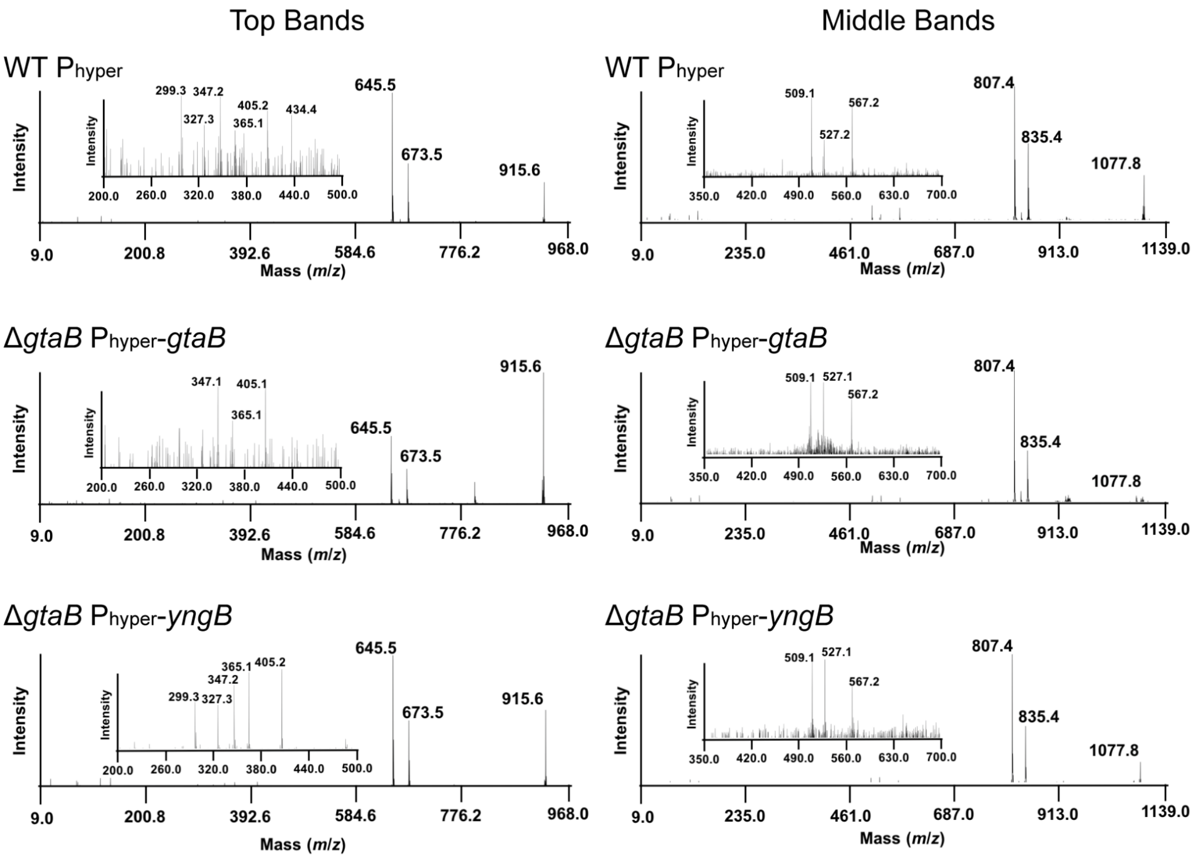

Figure S3. MALDI TOF MS/MS analysis of extracted glucolipids. Tandem mass spectrometry was performed on glycolipids isolated from one of the experiments from the wild-type *B. subtilis* (WT P_{hyper}) strain and the *gtaB* complementation strains Δ*gtaB* P_{hyper}-*gtaB* and Δ*gtaB* P_{hyper}-*yngB*. The analysis was performed on the glycolipids with an observed mass of 915.6 (*m*/*z*) (top band) and proposed to correspond to (32:0) [DAG-Glc_2 + Na^+] and an observed mass of 1077.9 (*m*/*z*) (middle band) and proposed to correspond to (32:0) [DAG-Glc_3 + Na^+]. The MS/MS spectra are shown in the figure and chemical formula for the fragmented species are listed in Table S1. The insert is an enlarged view of the species in the 200-550 *m*/z range for the glycolipids isolated from the top band or 350-700 *m*/*z* range for the glycolipids isolated from the middle bands.

References

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