**Supporting Information (SI)**

# Accumulation of heptaprenyl diphosphate sensitizes *Bacillus subtilis* to bacitracin: Implications for the mechanism of resistance mediated by the BceAB transporter

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short title: heptaprenyl diphosphate sensitizes *Bacillus subtilis* to bacitracin

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**Table S1:** Bacterial strains, plasmids, and oligos used in this study.[a]

| Strain | Genotype or description | Source, reference, or construction[1] |
|---|---|---|
| ***B. subtilis* strains** | | |
| 168 | *trpC2* | Lab Stock |
| CU1065 | *trpC2 attSPβ* | Lab Stock |
| HB0800 | NCBI3610 *(trp*$^{+}$*)* | Lab Stock |
| HB0106 | CU1065 *bcrC-pMUTIN* (MLS) | (Cao and Helmann, 2002) |
| HB0928 | 168 *bceAB::kan* | (Mascher *et al.*, 2003) |
| HB13129 | 168 *ytpA::MLS* | LFH PCR --> 168 |
| HB13320 | 168 *amyE*::$P_{spac(hy)}$*-ytpB* (*cat*) | pTK083 --> 168 |
| HB13321 | 168 *ytpB*::MLS | LFH PCR --> 168 |
| HB13346 | 168 *ytpB*::MLS *amyE*::$P_{spac(hy)}$*-ytpB* (*cat*) | chrDNA HB13320 --> HB13321 |
| HB13348 | 168 *bcrC-pMUTIN* (MLS) | chrDNA HB0106 --> 168 |
| HB13350 | 168 *ytpB*::*spec* | ECE79 --> HB13321 |
| HB13351 | 168 *ytpB*::*spec bcrC-pMUTIN* (MLS) | chrDNA HB0106 --> HB13350 |
| HB13354 | 168 *ytpB*::*spec bceAB*::*kan* | chrDNA HB0928 --> HB13350 |
| HB13355 | 168 *bcrC-pMUTIN*(MLS) *bceAB*::*kan* | chrDNA HB0928 --> HB13348 |
| HB13356 | 168 *ytpB*::*spec bcrC-pMUTIN* (MLS) *bceAB*::*kan* | chrDNA HB0928 --> HB13351 |
| HB13358 | 168 *sqhC*::MLS | LFH PCR --> 168 |
| HB13360 | 168 *sqhC*::MLS *ytpB*::*spec* | HB13358 chrDNA --> HB13350 |
| HB13367 | 168 *amyE*::$P_{bceA}$*-lacZ* (*cat*) | pTK099 --> 168 |
| HB13369 | 168 *amyE*::$P_{bceA}$*-lacZ* (*cat*) *ytpB*::*spec* | pTK099 --> HB13350 |
| HB13387 | 168 *trp*$^{+}$ | chrDNA HB800 --> 168 |
| HB13388 | 168 *bceAB::kan trp*$^{+}$ | chrDNA HB800 --> HB0928 |
| HB13389 | 168 *ytpB*::*spec trp*$^{+}$ | chrDNA HB800 --> HB13350 |
| HB13390 | 168 *ytpB*::*spec bceAB*::*kan trp*$^{+}$ | chrDNA HB800 --> HB13354 |
| HB13397 | 168 *amyE*::$P_{spac(hy)}$*-bceAB* (*cat*) | pTK102 -->168 |
| HB13398 | 168 *amyE*::$P_{spac(hy)}$*-hepSmenHhepT* (*cat*) | pTK103 -->168 |
| HB13399 | 168 *amyE*::$P_{spac(hy)}$*-menA* (*cat*) | pTK104 -->168 |
| HB13401 | 168 *amyE*::$P_{spac(hy)}$*-bceAB* (*cat*) *ytpB*::*spec* | chrDNA HB13397 --> HB13350 |
| HB13402 | 168 *amyE*::$P_{spac(hy)}$*-hepSmenHhepT (cat) ytpB*::*spec* | chrDNA HB13398 --> HB13350 |
| HB13403 | 168 *amyE*::$P_{spac(hy)}$*-menA (cat) ytpB*::*spec* | chrDNA HB13399 --> HB13350 |
| HB13438 | *menA*::*kan* | LFH-PCR --> 168 |
| HB13439 | *ytpB*::MLS *menA*::*kan* | HB 13438 chrDNA --> HB13321 |
| HB13443 | 168 *uppS1* (*kan-SD*-uppS*) *ytpB*::MLS | HB13321 chrDNA --> HB13648 |
| HB13447 | 168 *hepT*::*kan* | LFH-PCR --> 168 |
| HB13448 | 168 *ytpB*::MLS *hepT*::*kan* | HB13446 chrDNA --> HB13447 |
| HB13648 | 168 *uppS1* (kan-SD*-uppS) | (Lee and Helmann, 2013) |
| HB13649 | 168 *uppS1* (*kan-SD*-uppS*) *amyE::*$P_{spac(hy)}$*-uppS* | (Lee and Helmann, 2013) |
| HB17031 | 168 *bcrC-pMUTIN* (MLS) *trp*$^{+}$ | chrDNA HB800 --> HB13348 |

| | | |
|---|---|---|
| HB17032 | 168 *bcrC-pMUTIN*(MLS) *bceAB*::*kan trp*$^+$ | chrDNA HB800 --> HB13355 |
| HB17033 | 168 *ytpB*::*spec bcrC-pMUTIN* (MLS) *trp*$^+$ | chrDNA HB800 --> HB13351 |
| HB17034 | 168 *ytpB*::*spec bcrC-pMUTIN* (MLS) *bceAB*::*kan trp*$^+$ | chrDNA HB800 --> HB13356 |
| HB17055 | 168 *amyE*::$P_{spac(hy)}$-*uppS (cat)* | chrDNA HB13649 --> 168 |
| HB17057 | *168 ytpB*::*spec amyE*::$P_{spac(hy)}$-*uppS (cat)* | chrDNA HB17055 -->HB13350 |

| Plasmids | Genotype or description | Source |
|---|---|---|
| ECE76 | Vector for integration of a *cat* resistance cassette into a MLS resistance cassette | (Steinmetz and Richter, 1994) |
| ECE79 | Vector for integration of a *spec* resistance cassette into a MLS resistance cassette | (Steinmetz and Richter, 1994) |
| pdg1661 | Vector for integration of *lacZ* fusions at *amyE* | (Guerout-Fleury *et al.*, 1996) |
| pPL82 | Vector for IPTG-inducible control of gene expression with integration at the *amyE* locus | (Quisel *et al.*, 2001) |
| pTK083 | pPL82-ytpB | This work |
| pTK099 | pDG1661-$P_{bceA}$ *(cat)* | This work |
| pTK102 | pPL82-*bceAB* (*cat*) | This work |
| pTK103 | pPL82-*hepSmenHhepT* (*cat*) | This work |
| pTK104 | pPL82-*menA* (*cat*) | This work |

| Oligos | Name | Sequence |
|---|---|---|
| 5170 | ytpA-up-fwd | GAGAACAGGAAAGAATAGGT |
| 5171 | ytpA-up-rev (MLS) | GAGGGTTGCCAGAGTTAAAGGATCACAATCACGGCAACAGGTCT |
| 5172 | ytpA-do-fwd (MLS) | CGATTATGTCTTTTGCGCAGTCGGCAATGAACCCGAGAGAGAAGAT |
| 5173 | ytpA-do-rev | AGGCTGGAACGAATACGCAA |
| 5856 | ytpB-up-fwd | TCATGTGGACCTGGAAAGCA |
| 5857 | ytpB-up-rev (MLS) | GAGGGTTGCCAGAGTTAAAGGATCGTCCAGCTCTTGATGAACGA |
| 5858 | ytpB-do-fwd (MLS) | CGATTATGTCTTTTGCGCAGTCGGCGCGGCTTGCTTGGCGTTTA |
| 5859 | ytpB-do-rev | TGATCGTCCACCGCATTACA |
| 5860 | ytpB up XmaI | TCAGCCCGGGGAACCCGAGAGAGAAGATGT |
| 5861 | ytpB do XbaI | AGTCTCTAGATCAATTACGACAAACGGCAGT |
| 5944 | PbceA Forward EcoRI | GATCGAATTCGAACATGTCATAAGCGTGTGACG |
| 5945 | PbceA Reverse BamHI | GATCGGATCCTATCGATGCCCTTCAGCACTTCC |
| 5958 | sqhC upstream F | GCCTTTACCGGTTCCACTGA |
| 5959 | sqhC upstream R | GAGGGTTGCCAGAGTTAAAGGATCCGCCTCACTTTCTCCTGAAGT |
| 5960 | sqhC downstream F MLS | CGATTATGTCTTTTGCGCAGTCGGCGGAGACAGCAAATGAAACGTGA |
| 5961 | sqhC downstream R | CCACGACATTCCACCAGTGA |
| 5977 | hepST UbiE F XmaI | GATCCCCGGGTGTGAATTTGGGGACAAGGGT |
| 5978 | hepST UbiE R XbaI | GATCTCTAGATTAATCAATCCGGGGCGGAG |

| | | |
|---|---|---|
| 5979 | bceAB F XmaI | GATCCCCGGGTATCCAATATAAAGGAGACTGCGT |
| 5980 | bceAB R XbaI | GATCTCTAGAACAACCGATCCGGCTGAAAT |
| 5983 | menA F XmaI | GATCCCCGGGTTTTAATAGAAAGGAAGAGGAA |
| 5984 | menA R XbaI | GATCTCTAGACAAAATGCGGTCGATTGTAA |
| 6132 | menA up fwd | CCGTACACAAGGATAGGAGA |
| 6133 | menA up rev (kan) | CCTATCACCTCAAATGGTTCGCTGGCCAAAGGATCTGCCCCAT |
| 6134 | menA do fwd (kan) | CGAGCGCCTACGAGGAATTTGTATCGCTGGGTTGTCGGCTTGGTT |
| 6135 | menA do rev | GAAGGCGAAAGCATCTGACA |
| 6136 | hepT up fwd | CGGCTTATGAACGGAAATGAT |
| 6137 | hepT up rev (kan) | CCTATCACCTCAAATGGTTCGCTGCATCTGTGTTTTCATTTCCATC |
| 6138 | hepT do fwd (kan) | CGAGCGCCTACGAGGAATTTGTATCGTATGGCAGTAAGCGAAATGTAT |
| 6139 | hepT do rev | CGTTGGGAAAGGCAGAGGT |

[a]Abbreviations used: chrDNA indicates chromosomal DNA, LFH-PCR indicates long-flanking homology PCR, --> indicates genetic transformation.

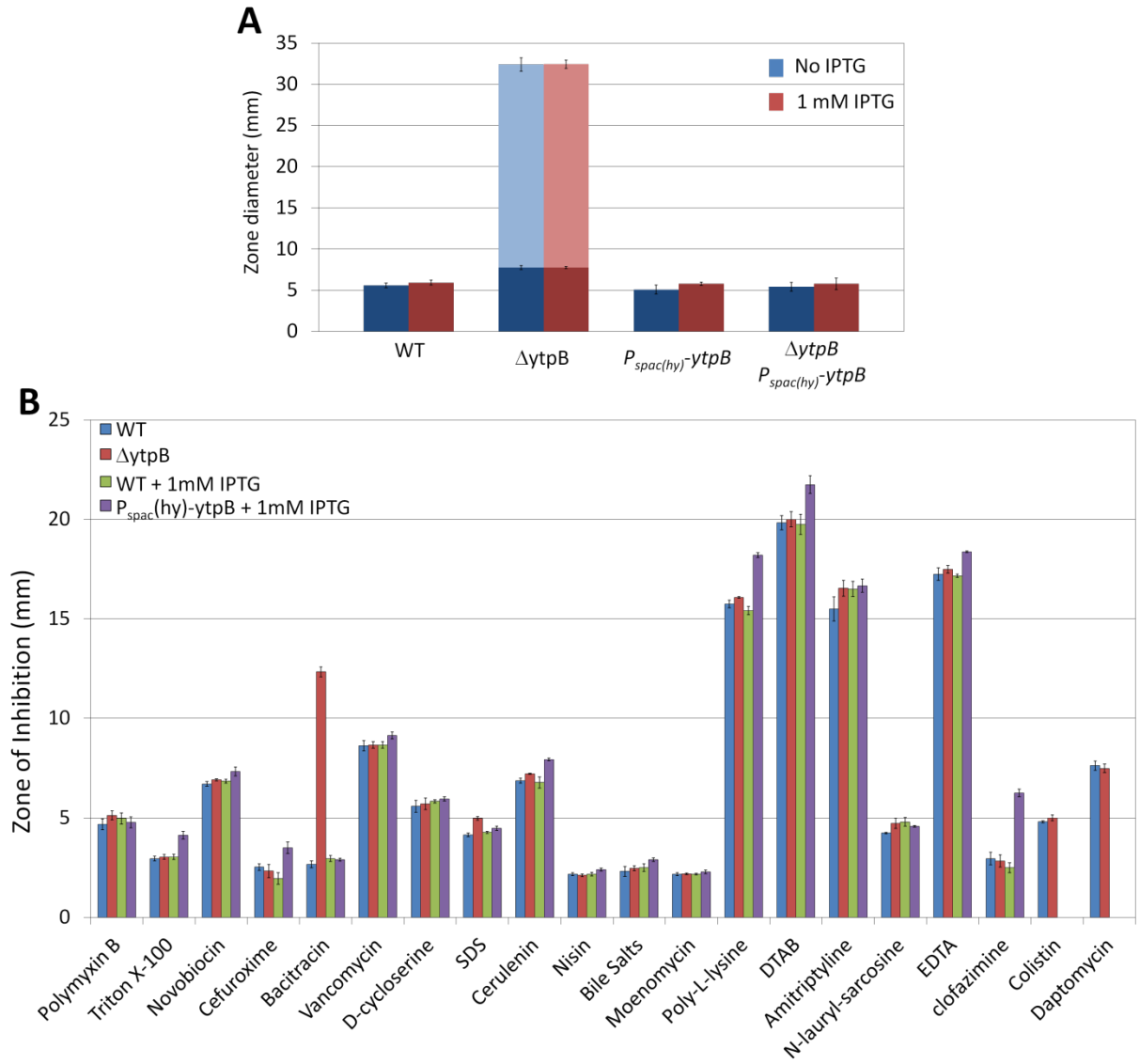


**Figure S1:** *ytpB* primarily confers resistance against bacitracin. (A) Bacitracin sensitivity of the WT (168), Δ*ytpB* (HB13321), $P_{spac}$*(hy)-ytpB* (HB13320), and $P_{spac}$*(hy)-ytpB* Δ*ytpB* (HB13346) strains as measured with a disk diffusion assay. Bacitracin sensitivity in the Δ*ytpB* background reverts to wild type upon the introduction of the $P_{spac}$*(hy)-ytpB* fusion even in the absence of IPTG induction. (B) The WT (168), Δ*ytpB* (HB13129), and $P_{spac(hy)}$*-ytpB* (HB13320) strains were assessed for sensitivity to the indicated stresses on MH media supplemented with or without 1 mM IPTG. The *ΔytpB* strain exhibited a large sensitivity phenotype to bacitracin (seen as a zone of early lysis; see text), but was not significantly more sensitive to the other stresses tested. Small but significant sensitivity phenotypes were observed for the $P_{spac}$*(hy)-ytpB* strain to triton X-100, cefuroxime, cerulenin, poly-L-lysine, dodecyltrimethylammonium bromide (DTAB), EDTA, and clofazamine.

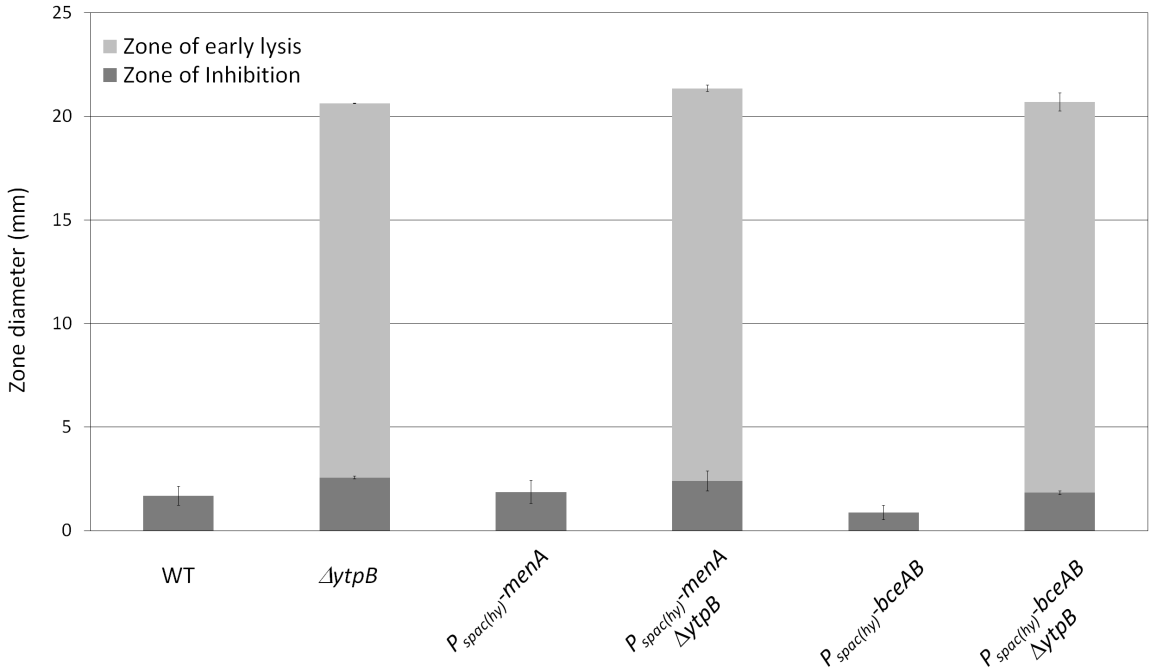


**Figure S2:** Bacitracin disk diffusion assays of WT (168), Δ*ytpB* (HB13350), $P_{spac(hy)}$-*menA* (HB13399), $P_{spac(hy)}$-*menA* Δ*ytpB* (HB13403), $P_{spac(hy)}$-*bceAB* (HB13397), and $P_{spac(hy)}$-*bceAB* Δ*ytpB* (HB13401) strains on MH medium supplemented with 1 mM IPTG. For this assay, bacitracin was used instead of bacitracin zinc. Overexpressing *menA* has no significant effect on bacitracin sensitivity. Overexpressing *bceAB* slightly decreased the bacitracin zone of inhibition in WT cells but did not affect sensitivity in Δ*ytpB* cells.

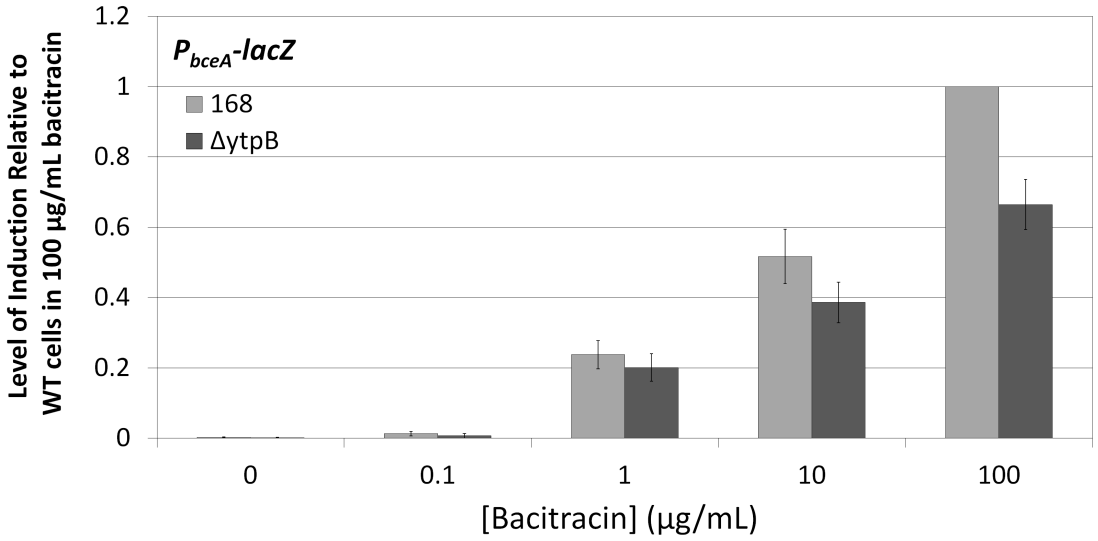


**Figure S3**. Induction of expression of *bceAB* by bacitracin in WT and Δ*ytpB* backgrounds. β-galactosidase assays were used to measure the activity of a $P_{bceA}$-*lacZ* construct in WT (HB13367) and Δ*ytpB* (HB13369) cells with bacitracin concentrations ranging from 0.1-100 µg $ml^{-1}$.

References

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