

Supplementary Information

Table S1 Primers used in this study

Description	Sequence (5' to 3')
5'- <i>kanR</i>	CAGACCGCTTCTGCGCAGTGGGCTTACATG
<i>kanR</i> -3'	GACGGCCAGTGAATTGAAATCTCGTGATGG
5'- <i>rrnB</i> terminator	CGCAGAAGCGGTCTGATAAAACAG
<i>rrnB</i> terminator-3'	GGAGAGCGTTCACCGACAAACAAC
5'- <i>araC</i> P _{BAD}	TGATTACGCCAAGCTTGTTAGCCCAAAAAACGG
<i>araC</i> P _{BAD} -3'	CGGTGAACGCTCTCCCAATTGTCTGATTCG
5'- <i>ybeX</i> P _{BAD} - <i>Int</i> integration	GTCAAAATCCCGGATGACTCACCCAGCCGAAGCTGGATGAATAAGAAATCTCGTGATGGCAGGTTGGGC
<i>ybeX</i> P _{BAD} - <i>Int</i> integration-3'	TTCAATTAATGAGGCAAAGCCATGTAGTTATCTATCCAGTTTCGGGTTAGCCCAAAAAACGGGTATGGAG
5'- <i>lpp::catR</i>	CTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCTATGAATATCCTCCTTAG
<i>lpp::catR</i> -3'	GCGCACAATGTGCGCCATTTTTCACTTACAGGTAATATTAGTAGGCTGGAGCTGCTTC
5'- <i>gutA::P_{BAD}-Int</i>	CATGGTGCAGAGTGGTTTATCGGGCTGTTCCAAAAGGGCGGAGAGGTGTTTAGACCATGATTACGCCAAGC
<i>gutA::P_{BAD}-Int</i> -3'	CGATCCCGGCGGATATCGTCCGGGACAGGGCCTGCCACATGGACAGTGCCCGTAAAACGACGGCCAGTG
5'- <i>chiQ::aprR</i>	CTCATCGCCATAATGGCATCGGGGCTGGTAGCTTGTCGCAATCACCTAGATCCTTTTGG
<i>chiQ::aprR</i> -3'	CTGCGTATTTCTGCCAGACTTTATCACAATCGGCTTTACCCGTTCTCCGCTCATGAGC
5'- <i>Int::specR</i>	GCCTCATTAATTGAACGCCAGCGCATTGCGCTGCTGCTGGCGATTCCCTGCTCGCGCAG
<i>Int::specR</i> -3'	CACAGCAGCAAACCAAACAATGCCGTGACACCCACAGCGGGCTTGAACGAATTGTTAG
5'- <i>Int</i> pUC19	GATTACGCCAAGCTTAGCCGAAGCTGGATG
<i>Int</i> pUC19-3'	GACGGCCAGTGAATTCATGTATTCCGGCAGC
5'- <i>lppK58A</i>	GCTTTTGTGAATTAATTTGTATATCG
<i>lppK58A</i> -3'	CACTTACAGGTAATATTACGCGCGGTATTTAGTAG
5'- <i>kanR</i>	CCTCGACTTCGCTGCAAGATCCCCTCACGCTG
<i>kanR</i> -3'	AGTACCTGTGAAGTGAAGCTTTTGGTCGGTCATTTTCG
5'- <i>repA</i>	GCAGCGAAGTCGAGGCATTTCTGTC
<i>repA</i> -3'	TTAATTCACAAAAGCGAATTCCTCGACAGTAAG
5'- <i>specR</i>	CAGGGGATCAAGATCTATTCCCCTGCTCGCGCAG
<i>specR</i> -3'	CTGTGAAGTGAAGCTTGCTTGAACGAATTGTTAG
5'- <i>lppK58A-Strep</i>	CATCCTCAATTTGAAAAATAATAGTACCTGTGAAGTG
<i>lppK58A-Strep</i> -3'	ACTCCACGCTGAACCGGATCCCAGCGCGGTATTTAGTAG
5'-pKFC WMC_RS08810 upstream	GACGGCCAGTGAATTCAGCCAGTGATTCG
pKFC WMC_RS08810 upstream-3'	GAGTCCAAGTGTTCCTTAAACGCAATTTTCG
5'-WMC_RS08810 downstream	GAAACACTTGGACTCTTATTAGAAGGCCTG
WMC_RS08810 downstream-3'	TGATTACGCCAAGCTCATCTAGTCTTCGG
5'-pKFC BC1526 upstream	GACGGCCAGTGAATTTCTGTGGCAACTGG
pKFC BC1526 upstream-3'	CCATTGTTGCGAGTGCAAAAATAGAAAATGC

5'-pKFC BC1526 downstream	GCACTCGCAACAATGGTCTCTGCTTAAAGAG
pKFC BC1526 downstream-3'	TGATTACGCCAAGCTAGGCACTACGTACTC
5'-WMC_RS08810 pMSP3535	CCACTAGTCCC GGGCTGCAGCCTATGTTGTTGATTCC
WMC_RS08810 pMSP3535-3'	GAGACCGGCCTCGAGGCGAGCAATTTTGAATTG
5'-BC1526 pSP-hp	CAAGGAGGTGAATGTACATTGGATCGATTGATTAC
BC1526 pSP-hp-3'	CGGCCGGTACCGGATCCTAGAACAGAACTAGACCG

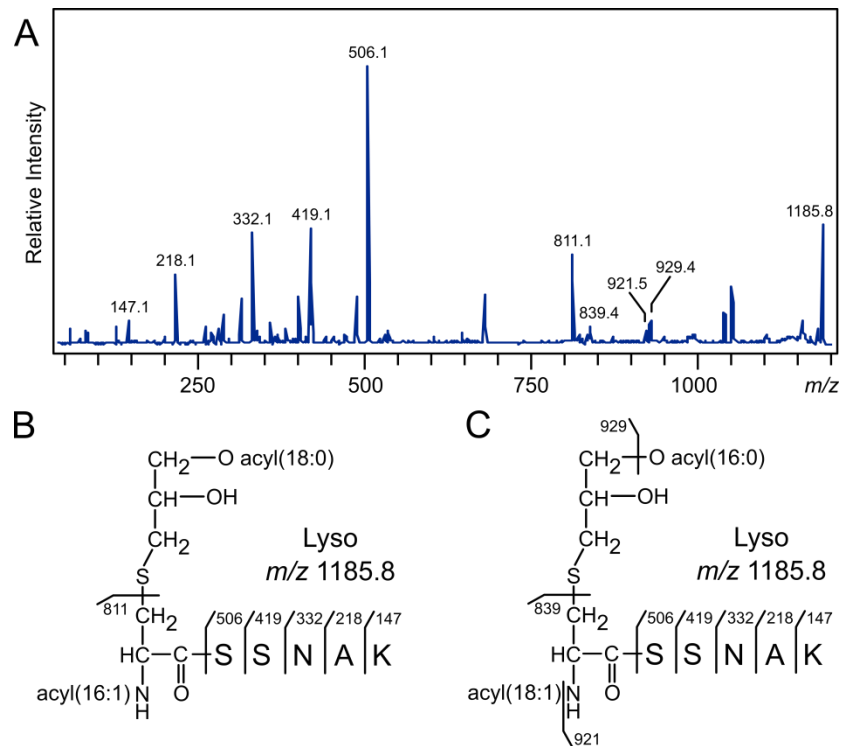


FIG S1 MALDI-TOF MS/MS Spectrum of the *E.coli* Lpp ion m/z 1185. MS/MS spectrum of the m/z 1185 ion of Lpp purified from KA532 (A) and the two elucidated structures (B and C) of lyso-form Lpp featuring lipidated cysteine with two fatty acids totaling 34:1.

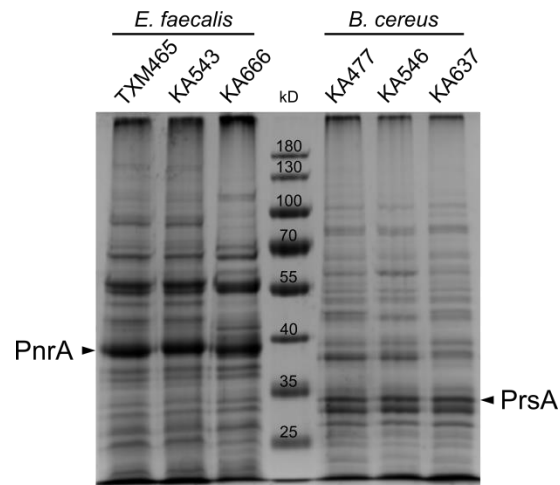


FIG S2 Lipoproteins from *E. faecalis* and *B. cereus*. Using the Triton X-114 phase partitioning method, lipoproteins were enriched from *E. faecalis* strains TXM465 (WT), KA543 (Δ WMC_RS08810), and KA666 (Δ WMC_RS08810 + pMSP3535-WMC_RS08810), along with *B. cereus* strains KA477 (WT), KA546 (Δ BC1526), and KA637 (Δ BC1526 + pSP-BC1526-hp). The resulting Triton X-114 phases were analyzed by SDS-PAGE and visualized by Coomassie blue staining.

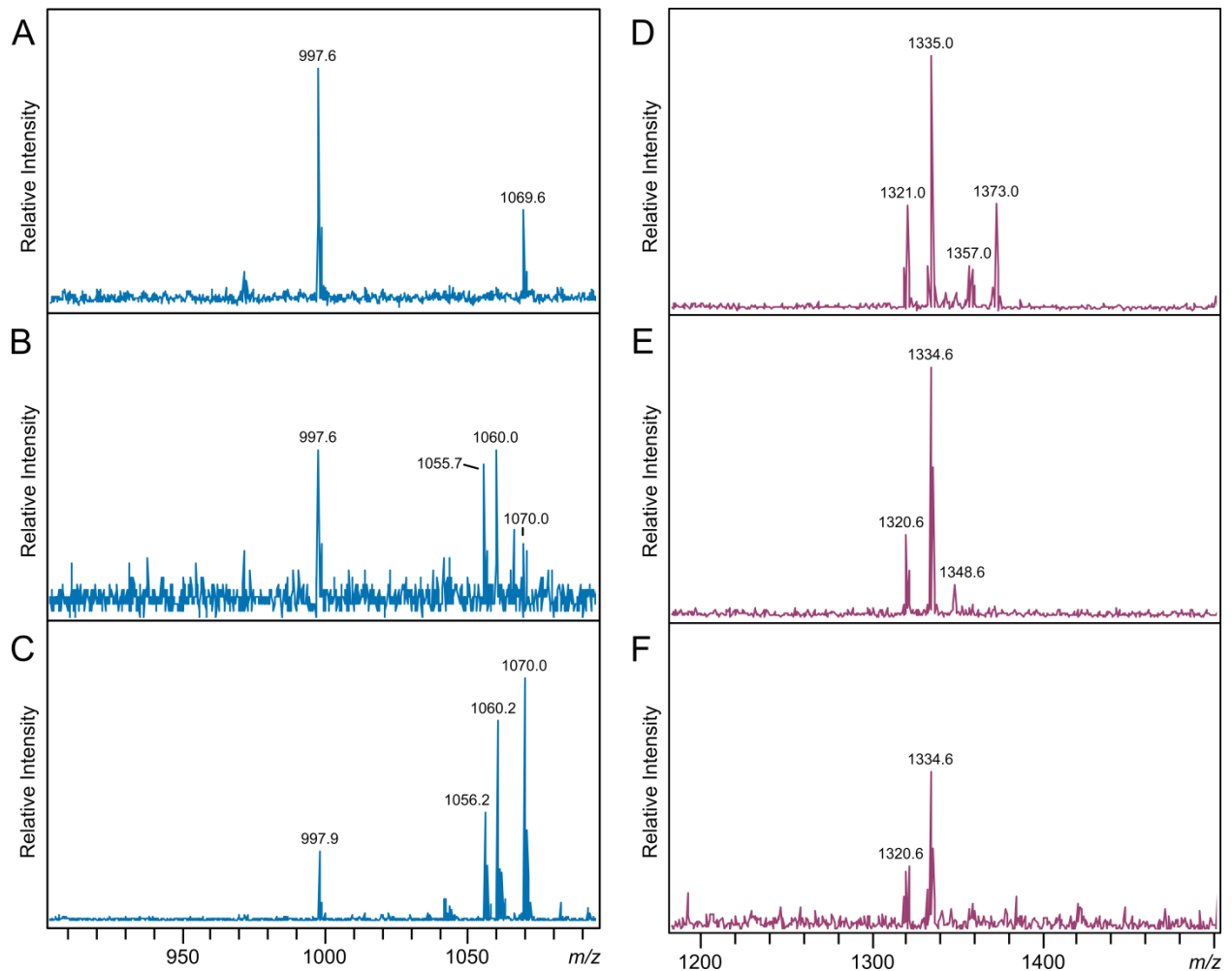


FIG S3 MALDI-TOF MS of *E. faecalis* PnrA and *B. cereus* PrsA. Parent MS spectra of the m/z 997 ion region corresponding to the *N*-terminal lipopeptide of *E. faecalis* PnrA purified from the wildtype strain (A), the deletion strain (B), and the back-complemented strain (C). MS spectra of the m/z 1334 parent ion region corresponding to the *N*-terminal lipopeptide of *B. cereus* PrsA purified from the wildtype strain (D), the deletion strain (E), and the back-complemented strain (F).