Supporting Information:

A peptidoglycan hydrolase of an unusual cross-link cleavage-specificity contributes to bacterial cell wall synthesis

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Supporting Materials and Methods

Plasmid constructions

For PCR amplifications, genomic DNA of MG1655 strain was used as a template unless otherwise indicated. Amplification of DNA was done using Phusion DNA polymerase (NEB) and clones obtained were confirmed by sequence analysis.

pPK2. The *mepK* gene along with its ribosome binding site (RBS) was PCR amplified using forward and reverse primers 5'-GCTCTAGATAGACTTGATTATCATGGACAAAT-3' and 5'-CCCAAGCTTGGCAATTACTACCAGTGCCGTGCT-3' respectively and the resulting amplified DNA fragment was cloned at XbaI-HindIII sites (underlined) of pTrc99a to obtain pPK2. This clone complemented the NA-sensitivity of the double mutant Δ*mepS* Δ*mepK* at 30°C with 0.5 mM IPTG.

pPK6. A fragment encoding MepK$^{31\text{-}182}$ was cloned into pET21b vector in between NdeI and XhoI sites (underlined) using forward and reverse primers 5'-GGAATTCCATATGACACTCTCTACCCCACGCCCG-3' and 5'-CCGCTCGAGCCAGTGCCGTGCTGGCCCGG-3' respectively, to generate a C-terminal 6XHis fusion construct.

pPK19, -20, -21. For generation of site directed variants of *mepK* (H133A, D140A and H173A), a 3 step PCR was performed. For this procedure, two primers were synthesized that are complementary to each other with desired mutations at the center (in bold and underlined). In the first step, N-terminal fragment of *mepK* gene was amplified using a common forward primer and a reverse primer containing the desired mismatch. In the second step, C-terminal fragment of *mepK* gene was amplified using a forward primer containing the desired mismatch and a common reverse primer. Desired mismatches code for an alanine instead of H133, D140 or H173 in MepK. Common forward primer (containing an optimal RBS and a XbaI site, in lower case and underlined, respectively) and reverse primer (6x His tag and a HindIII site, in lower case and underlined, respectively) are given below:

Common forward primer:
5'-TGCTCTAGAagggaagaattATGGACAAATTCGACGCTAATCGCCGC-3' and

Reverse primer:
5’-CCCAAGCTTTCAatggtgatggtgatggtgCCAGTGCCGTGCTGGCCCGGTATC-3’.

The forward and reverse primers used for nucleotide substitution are: for H133-

5’-CGTGGAGTAGCGAAGAAAAGCTAT**GCG**ACTAAAGGCCAGGCGATGGATTTC-3’ and 5’-GAAATCCATCGCCTGGCCTTTAGT**CGC**ATAGCTTTTCTTCGCTACTCCACG-3’;

D140- 5’-CACACTAAAGGCCAGGCGATGG**CG**TTCCATATTGAAGGTATCGCG-3’ and 5’-CGCGATACCTTCAATATGGAA**CG**CCATCGCCTGGCCTTTAGTGTG-3’;

H173- 5’-CCACGTAGTAACTTTGTG**GCG**ATTGATACCGGGCCAGCACG-3’ and 5’-CGTGCTGGCCCGGTATCAAT**CGC**CACAAAGTTACTACGTGG-3’.

In the third step, both the PCR products were mixed in 1:1 molar ratio and end filling was done by PCR for first 10 cycles at low annealing temperature. After addition of common forward and reverse primer, PCR was resumed for the next 30 cycles. The final PCR product was digested with XbaI and HindIII and cloned into pTrc99a digested with the same enzymes. The recombinant plasmids, pPK19 (*mepK*-H133A), pPK20 (*mepK*-D140A) and pPK21 (*mepK*-H173A) were confirmed for the presence of mutations by sequencing.

pPK17, -23, -25. MepK encoding fragments from three different organisms, *Escherichia coli, Caulobacter crescentus* (CCNA_01580) and *Klebsiella pneumoniae* (WP_004201403, DUF882) were PCR amplified using their respective genomic DNA as template with the corresponding forward and reverse primers. Forward and reverse primers of *E. coli* are:

5’-TGCTCTAGAagggaagaattATGGACAAATTCGACGCTAATCGCCGC-3’ and

5’-CCCAAGCTTTCAatggtgatggtgatggtgCCAGTGCCGTGCTGGCCCGGTATC-3’;

C. crescentus- 5’-TGCTCTAGAagggaagaattATGCATCGACGTAAGCTGCTCCAGTG-3’ and 5’-CCCAAGCTTTCAatggtgatggtgatggtgGGTCCCCGTCCACTTGCGGAC-3’;

K. pneumoniae- 5’-TGCTCTAGAagggaagaattATGGACAAATTTGACGCTAATCGCCGCA-3’ and 5’-CCCAAGCTTTCAatggtgatggtgatggtgCCAGTGCCTTACGGGACCGGTATC-3’.

The resulting amplified DNA fragments were cloned at XbaI-HindIII sites of pTrc99a to obtain pPK17 (pTrc99a *mepK*-His$_{E.\ coli}$), pPK23 (pTrc99a *mepK*-His$_{C.\ crescentus}$) and pPK25 (pTrc99a *mepK*-His$_{K.\ pneumoniae}$).

The clones encoding MepK of *E. coli* and *K. pneumoniae* complemented the NA-sensitivity of the double mutant Δ*mepS* Δ*mepK* at 30°C with 0.5 mM IPTG very well whereas complementation with MepK of *C. crescentus* was weak.

pPK29- MepK$^{32\text{-}183}_{K.\ pneumoniae}$ fragment was cloned into pET21b vector using NdeI and XhoI sites (underlined) using forward and reverse primers

5'-GGAATTCCATATGACCCTCTCGACCCCCCGTCCG-3' and

5'-CCGCTCGAGCCAGTGCCTTACGGGACCGG-3' respectively to generate a C-terminal 6XHis fusion vector.

Supplementary Methods:

Viability assays and Microscopy. To check viability of the strains, overnight grown cultures were serially diluted (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}) and 5 µL aliquots of each dilution were spotted onto the required plates followed by incubation at the indicated temperature. Generally, 16–20 h was the incubation time for LB and NA and 24–36 h for MM plates. For growth measurements, overnight grown culture of the indicated strains were diluted 1:100 in fresh medium and allowed to grow at the desired temperature. OD values at 600 nm were recorded at every 1 h interval and data were plotted using Origin software. Cell viability was measured using the LIVE/DEAD Baclight bacterial viability kit (Molecular Probes, Invitrogen). Immobilized cultures on a thin 1% agarose pad were visualized using a Zeiss apotome microscope by DIC (Nomarski optics) and fluorescence microscopy using GFP and DsRed filters.

Preparation of PG sacculi. Isolation of PG was done as described earlier (1). Cells of indicated strains grown to OD_{600} of 1.0 were harvested by centrifugation at 10,000×g for 10 min at 4°C. Cell pellet (from 1000 mL) was resuspended in 6 mL of ice-cold deionized water and added drop wise into 6 mL of boiling 8% SDS with vigorous stirring and then allowed to boil for another 45 min to completely solubilize membranes and to destroy high molecular weight DNA. The mixture was incubated overnight at room temperature and PG sacculi were collected by high speed centrifugation (200,000×g, 40 min). The pellet was washed several times with deionized water to remove SDS. High molecular weight glycogen and covalently bound lipoprotein were removed by treating with α-amylase (100 µg/mL in 10 mM Tris-HCl, pH 7.0, 2 h at 37°C) and pre-digested pronase (200 µg/mL, 90 min at 60°C) respectively. Enzymes were inactivated by boiling with equal volume of 8% SDS for 15 min. Pure PG sacculi were collected by ultracentrifugation and washed several times with water until SDS was completely removed. Concentration of SDS was checked as described earlier (2). The final pellet was resuspended in 0.5 mL of 25 mM Tris-HCl (pH 8.0) and stored at -30°C.

Analysis of PG sacculi. Analysis of PG sacculi was done as previously described (1). Soluble muropeptides were prepared by digesting the sacculi with 10 U mutanolysin (Sigma-Aldrich) at 37°C in 25 mM Tris-HCl (pH 8.0) for 16 h and insoluble material was removed by centrifugation. Soluble fraction was reduced with 1 mg of sodium borohydride in 50 mM sodium borate buffer (pH 9.0) for 30 min and excess borohydride was destroyed by addition of 20%

phosphoric acid. pH was adjusted to 3–4 and samples were loaded onto a reverse phase C18 column connected to Agilent technologies RRLC 1200 system. Column temperature was set at 55°C and binding was allowed at a flow rate of 0.5 mL/min with 1% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) for 10 min. Muropeptides were eluted in a gradient of 1–10% acetonitrile containing 0.1% TFA at a flow rate of 0.5 mL/min for next 60 min (using RRLC online software called Chemstation). Absorbance was detected at 205 nm.

Mass spectrometry (MS) analysis of muropeptides. The muropeptide peaks recovered from fractions of HPLC were dried and reconstituted into 5% acetonitrile with 0.1% formic acid and then loaded onto a reverse phase PepMap^{TM} RSLC C18 column (3µm, 100Å, 75µm x 15cm) connected to Q-Exactive^{TM} HF Hybrid Quadrupole-Orbitrap^{TM} Mass Spectrometer (Thermo Fisher Scientific, USA). Relevant peaks were analyzed by tandem MS and structures were deciphered based on molecular mass of the fragments.

Protein purification. MepK encoding plasmid, pET21b *mepK*$^{31-182}$ (pPK6) was transformed into C41 (λDE3) strain (3) and a single transformant was inoculated into 10 mL LB broth with Amp and grown overnight. The culture was diluted 1:100 into a fresh pre-warmed 1000 mL LB broth with Amp, allowed to grow until OD_{600} of ~0.6 before being induced by addition of 0.5 mM IPTG and grown further for 6 h at 18°C. Cells were recovered by centrifugation and pellet was stored at -80°C until further use. Cell pellet was resuspended in 20 mL lysis buffer (50 mM Tris, 500 mM NaCl, 20 mM imidazole, pH 8.0) and lysed by sonication (20% Amplitude; 10 sec on-off). Cell debris was removed by centrifugation at 30,000×g for 30 min at 4°C. Supernatant was mixed with 1 mL Ni^{2+}- NTA agarose (Qiagen) and mixed at 4°C for 1 h. This mixture was loaded into empty plastic columns (Bio-Rad) and washed with 100 mL wash buffer-I (50 mM Tris, 500 mM NaCl, 30 mM imidazole, 1% Triton-X-100, pH 8.0), 30 mL of wash buffer-II (50 mM Tris, 500 mM NaCl, 50 mM imidazole, 1% Triton-X-100, pH 8.0), 25 mL of wash buffer-III (50 mM Tris, 100 mM imidazole pH 8.0). Bound proteins were eluted with 30 mL of elution buffer (50 mM Tris, 250 mM imidazole, pH 8.0). The eluate was mixed with 1 mL of SP-sepharose (Amersham, cation exchange matrix) and mixed at 4°C for 1 h. The mixture was transferred to a column and washed with 10 mL buffer (50 mM Tris, 20 mM NaCl, pH 8.0). The bound proteins were eluted with a linear gradient of elution buffer (50 mM Tris, 50 mM-2.0 M NaCl, pH 8.0). Purified protein was pooled and concentrated to 2.5 mL using a 3 kD cut-off

centrifugal membrane filter (Millipore). This eluate was loaded on to a buffer exchange PD10 column (Amersham) and retained proteins were eluted in 3.5 mL storage buffer (100 mM Tris, 200 mM NaCl and 2 mM DTT). Protein was concentrated to 250 µL by 3 kD cut-off centrifugal membrane filter before being mixed with equal volume of 100% glycerol and stored at -30°C for further use.

$MepK^{32\text{-}183}_{K.\ pneumoniae}$ protein was purified using the plasmid pPK29 in the similar way as $MepK_{E.\ coli}$ except that the induction for protein overexpression was for 3h at 30°C.

Zymogram assay. Purified proteins were electrophoresed on two 12% SDS-gels in which one gel was impregnated with *Micrococcus lysodeikticus* cells. After electrophoresis, the gel with cells was incubated overnight in renaturation buffer (25 mM Tris-Cl, pH 8.0 and 1% Triton X-100) at RT. The gel was stained with methylene blue (0.1% methylene blue in 0.01% KOH) to visualize the zone of clearance. Another gel without cells was stained with Coomassie brilliant blue.

Western blotting. Samples were boiled with Laemmli loading dye and proteins were separated by SDS-PAGE. Primary α-His antibodies were used at 1:3000 dilution. Secondary anti-mouse-HRP conjugate antibodies were used at a dilution of 1:10000. For developing blot, ECL chemiluminescent detection reagents (GE biosciences) were used.

Supplementary Figure legends:

Fig. S1. Phenotypes of *mepK* mutant. (A) Panel shows microscopic images of WT and its various mutant derivatives. Indicated strains were grown overnight and diluted 1:100 into fresh LB broth and grown till A_{600} of 0.3 at 30°C. Cultures were pelleted, washed once, resuspended in Nutrient Broth (NB) and grown at 30°C. Cells were collected 2 h after the shift to NB. Live-Dead cell staining was done as described in SI materials and methods. Green and Red represent Live and Dead cells, respectively. DIC (panel I) and fluorescence (panel II) images are shown. Scale bar represents 5μm. (B) β-lactam-sensitivity of WT and its mutant derivatives was examined using the indicated strains. Cultures are grown overnight in LB broth, serially diluted and 5 μL of each dilution were spotted on indicated plates and grown overnight. Cephalexin was used at 5.5 μg/mL.

Fig. S2. Genetic interactions of *mepK* with *mepS* and *mepM*. (A) Cells of MR506 (BW27783 Δ*mepS* Δ*mepM*/pBAD33-*mepS*) harboring either pTrc99a (vector) or pPK2 (P_{trc}::*mepK*) were grown in LB broth containing 0.2% arabinose, washed to remove traces of arabinose, serially diluted and 5 μL of various dilutions were spotted on indicated plates. Arabinose, glucose and IPTG were used at 0.2 %, 0.2 % and 0.5 mM, respectively. (B) Cells of MR510 (MG1655 Δ*mepS* Δ*mepM*/pBAD33-*mepS*) and PC038 (MR510 Δ*mepK*) were grown in LB broth containing 0.2% arabinose. Cultures were washed with minimal medium to remove traces of arabinose, serially diluted and 5 μL of each dilution were spotted on minimal media (MM) plates supplemented with 0.2% arabinose or glucose. (C) Microscopy was done using the strains grown in minimal medium supplemented with 0.2% glucose. Cells were collected after 2 h of growth and visualized with DIC microscopy. Arrows indicate dead/lysed cells. Scale bar = 5 μm.

Fig. S3. PG composition of *mepA* deletion strains. PG sacculi were isolated from Δ*mepA* and Δ*mepA* Δ*mepK* mutant strains, digested with mutanolysin and the resulting soluble muropeptides were separated by RP-HPLC. Percentage area of relevant muropeptides is also shown.

Fig. S4. Zymogram assay showing MepK activity. Purified MepK (5 μg, lane 4) and MepM (10 μg, lane 5) were electrophoresed on a 12% SDS-gel containing *Micrococcus lysodeikticus* cells. The gel was renatured overnight in 25 mM Tris-HCl (pH 8.0) containing 1% Triton-X-100 and stained with methylene blue/KOH (gel-II). Another gel was run similarly and stained with

Coomassie brilliant blue (gel-I). Both gels have BSA (12 μg, lane 2) and lysozyme (8 μg, lane 3) as negative and positive controls, respectively.

Fig. S5. Activity of MepM on 3–3 cross-linked muropeptides. Soluble muropeptides (peptidoglycan sacculi of WT digested with mutanolysin) were treated with either buffer or 5 μM MepM and separated by RP-HPLC. MepM was active on peaks 3 and 5 but not on peak 4. MepM was purified as described earlier (4).

Fig. S6. Identification of purified muropeptides by mass spectrometry. Mass spectra of various muropeptides indicating the molecular mass (both +1 and +2 charges) is shown along with their structures.

Fig. S7. Activity of MepK on intact PG sacculi. Intact PG sacculi of WT were treated with either buffer or 5 μM MepK for 20 h at 30°C, heat-inactivated for 30 min at 90°C followed by mutanolysin treatment for 16 h and separated by RP-HPLC. Note that MepK completely cleaved 3–3 cross-linked Tri-Tetra (peak 4) and partially cleaved 4–3 cross-linked Tetra-Tetra (Peak 5) muropeptides.

Fig. S8. Functionality and stability of MepK mutants and orthologs. (A) Three-dimensional structure of MepK made by Swiss-model (homology-modeling server) using a highest scoring template, Zinc D-ala–D-ala carboxypeptidase of *Streptomyces albus G* (Protein Data Bank ID: 1LBU). Zinc-binding amino acid residues predicted by the model, Histidine (H) at position 133 and 173 (blue); Aspartic acid (D) at 140 (red); and Zinc cation (black) are marked. (B) HPLC chromatograms of Δ*mepK* mutant carrying vector (pTrc99a) or pPK17 (P_{trc}::*mepK*-$His_{E.\ coli}$), pPK20 (P_{trc}::*mepK*-His_{D140A}) or pPK25 (P_{trc}::*mepK*-$His_{K.\ pneumoniae}$). Cultures were grown up to 1 OD in LB broth containing 0.5 mM IPTG, PG sacculi isolated, solubilized and separated by RP-HPLC. Red arrows represent peak 4 (Tri-Tetra). (C) Cultures of WT harboring pPK17 (P_{trc}::$mepK_{E.\ coli}$-His), pPK19 (P_{trc}::*mepK*-His_{H133A}), pPK20 (P_{trc}::*mepK*-His_{D140A}) or pPK21 (P_{trc}::*mepK*-His_{H173A}) were grown in LB broth containing 0.5 mM IPTG and cell lysates were subjected to western blot analysis using anti-His antibodies. (D) WT carrying vector (pTrc99a) or pPK17 (P_{trc}::*mepK*-$His_{E.\ coli}$), pPK23 (P_{trc}::*mepK*-$His_{C.\ crescentus}$) or pPK25 (P_{trc}::*mepK*-$His_{K.\ pneumoniae}$) were grown in LB broth containing 0.5 mM IPTG and western blot analysis was done as described above. MW of $MepK_{E.\ coli}$ is 20.35 kDa; $MepK_{K.\ pneumoniae}$ is 20.48 kDa and $MepK_{C.}$

crescentus is 23.59 kDa. (E) Soluble muropeptides of WT *E. coli* were incubated either with buffer or $MepK^{32\text{-}183}_{Kp}$ protein (5 µM) for 20 h and separated by RP-HPLC. $MepK_{Kp}$ completely cleaved Tri-Tetra muropeptides (red arrow). *Kp* represents *Klebsiella pneumoniae.* However, recombinant $MepK^{26\text{-}216}$ of *Caulobacter crescentus* could not be purified due to its poor solubility.

Supplementary Figures:

Fig. S1

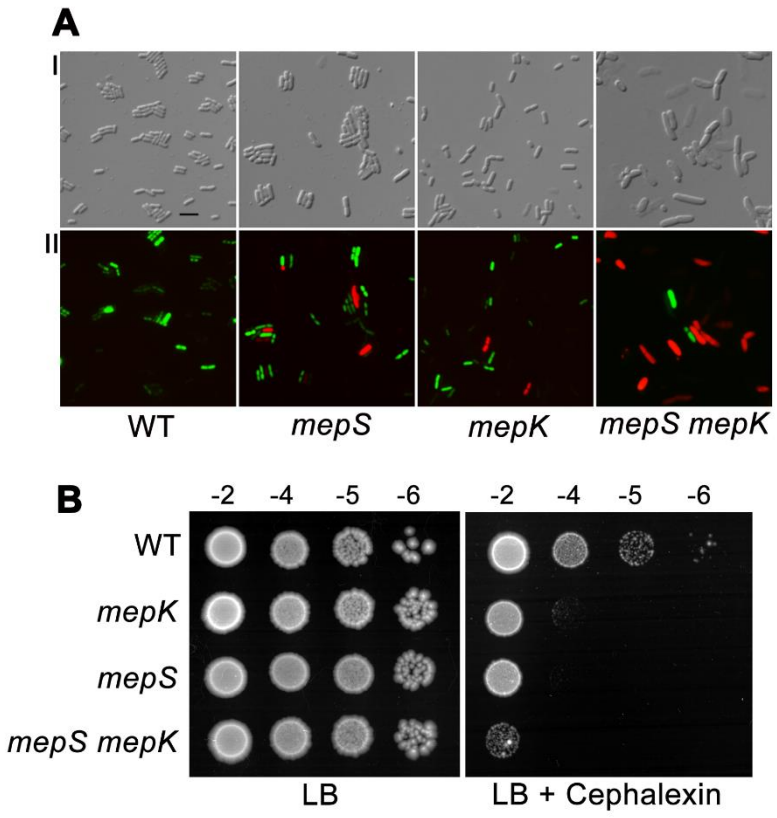
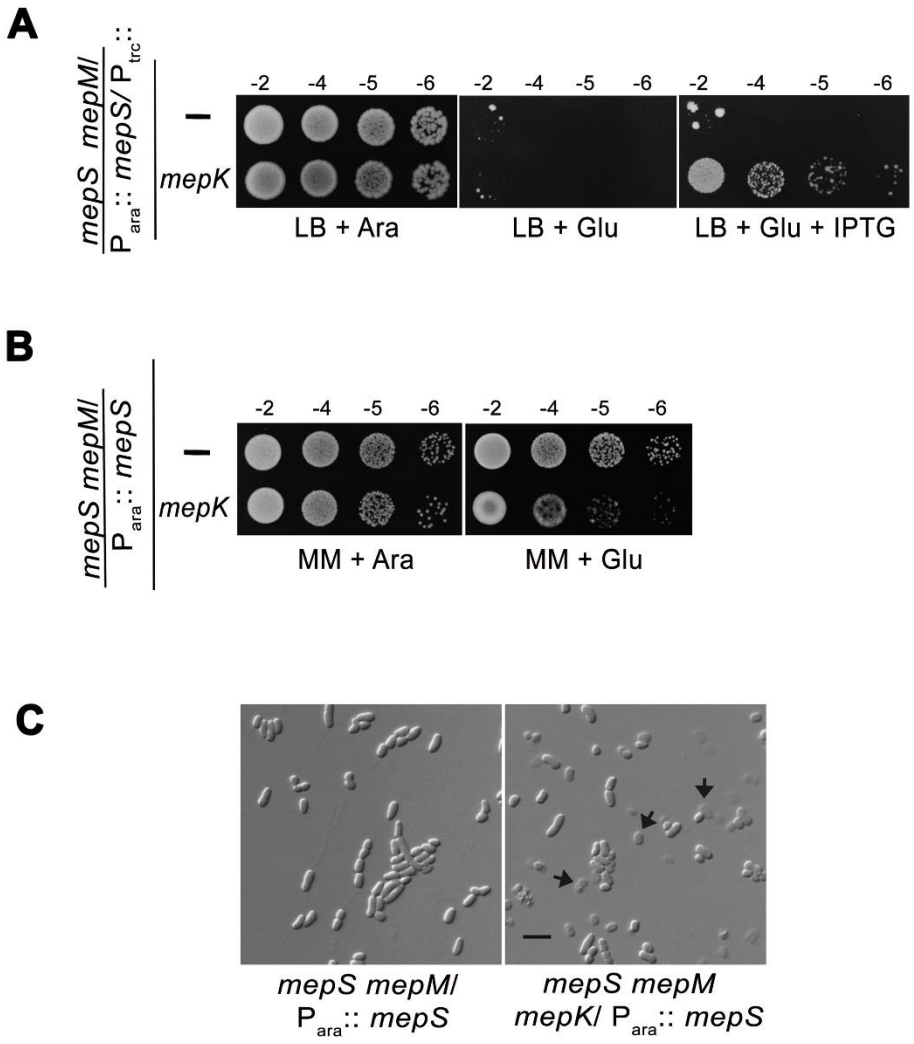


Fig. S2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mepK
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LB + Ara
LB + Glu
LB + Glu + IPTG
B
mepS mepM/ P_ara:: mepS
–
mepK
-2 -4 -5 -6 -2 -4 -5 -6
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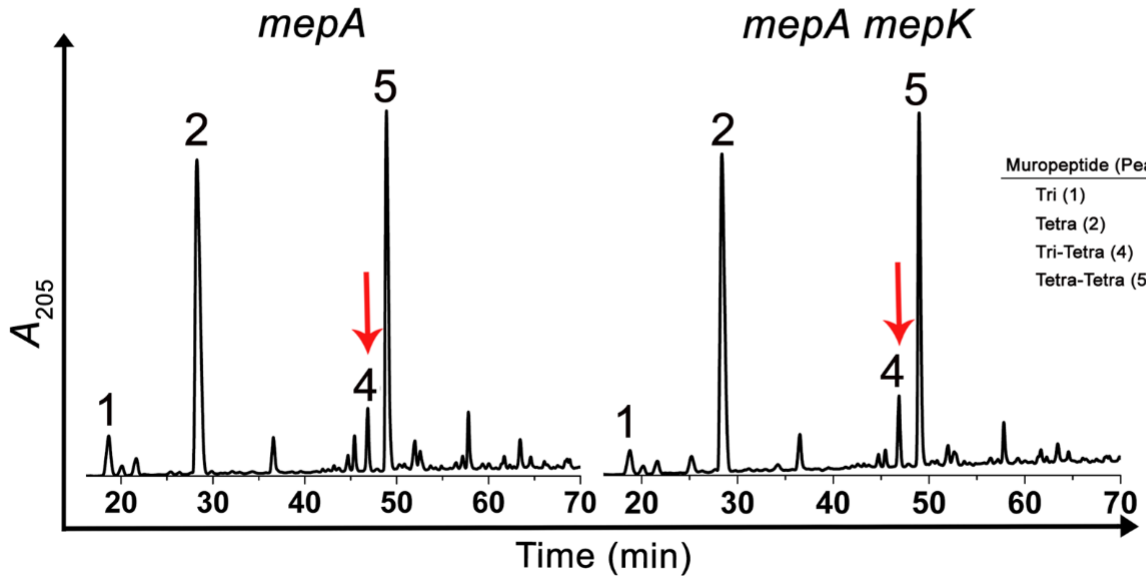
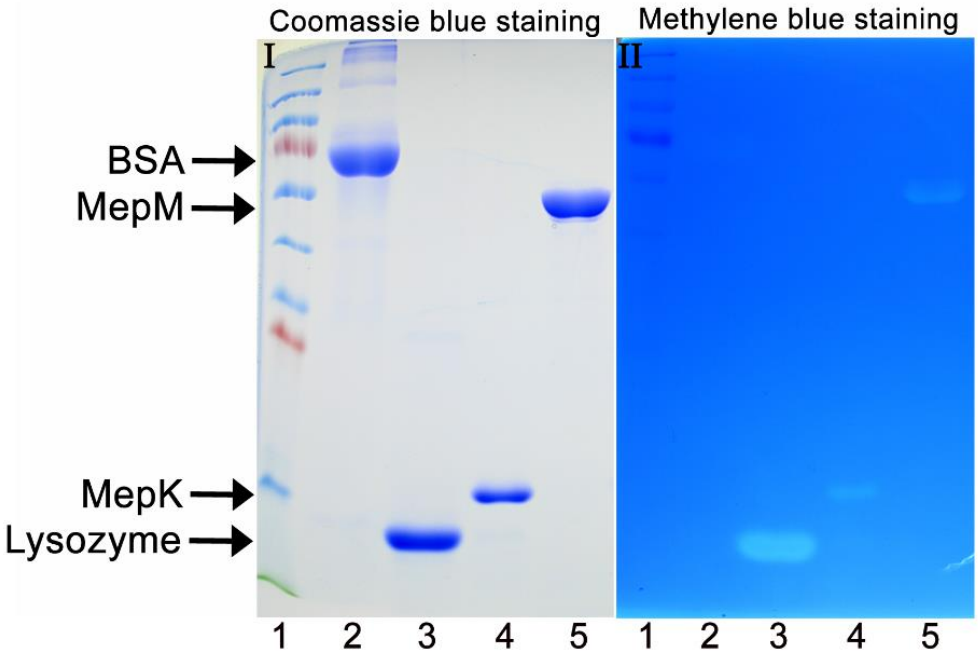
mepS mepM mepK/ P_ara:: mepS

Fig. S3



Muropeptide (Peak)	% Area of muropeptides	
	mepA	*mepA mepK*
Tri (1)	4.53 ± 0.78	3.27 ± 1.00
Tetra (2)	44.58 ± 0.18	45.29 ± 1.08
Tri-Tetra (4)	4.46 ± 0.18	6.27 ± 0.52
Tetra-Tetra (5)	31.41 ± 0.89	28.99 ± 2.82

Fig. S4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MepM
MepK
Lysozyme
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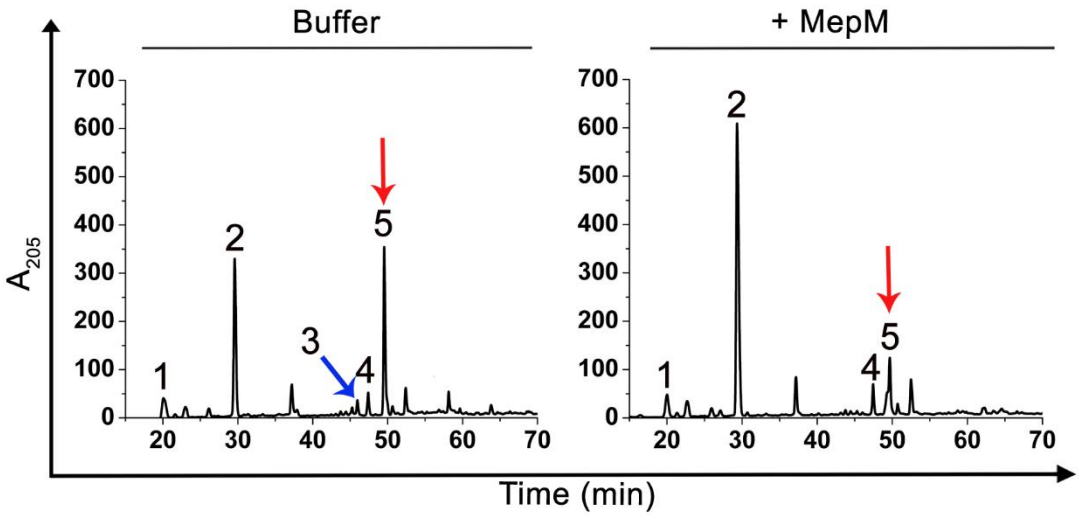
Fig. S5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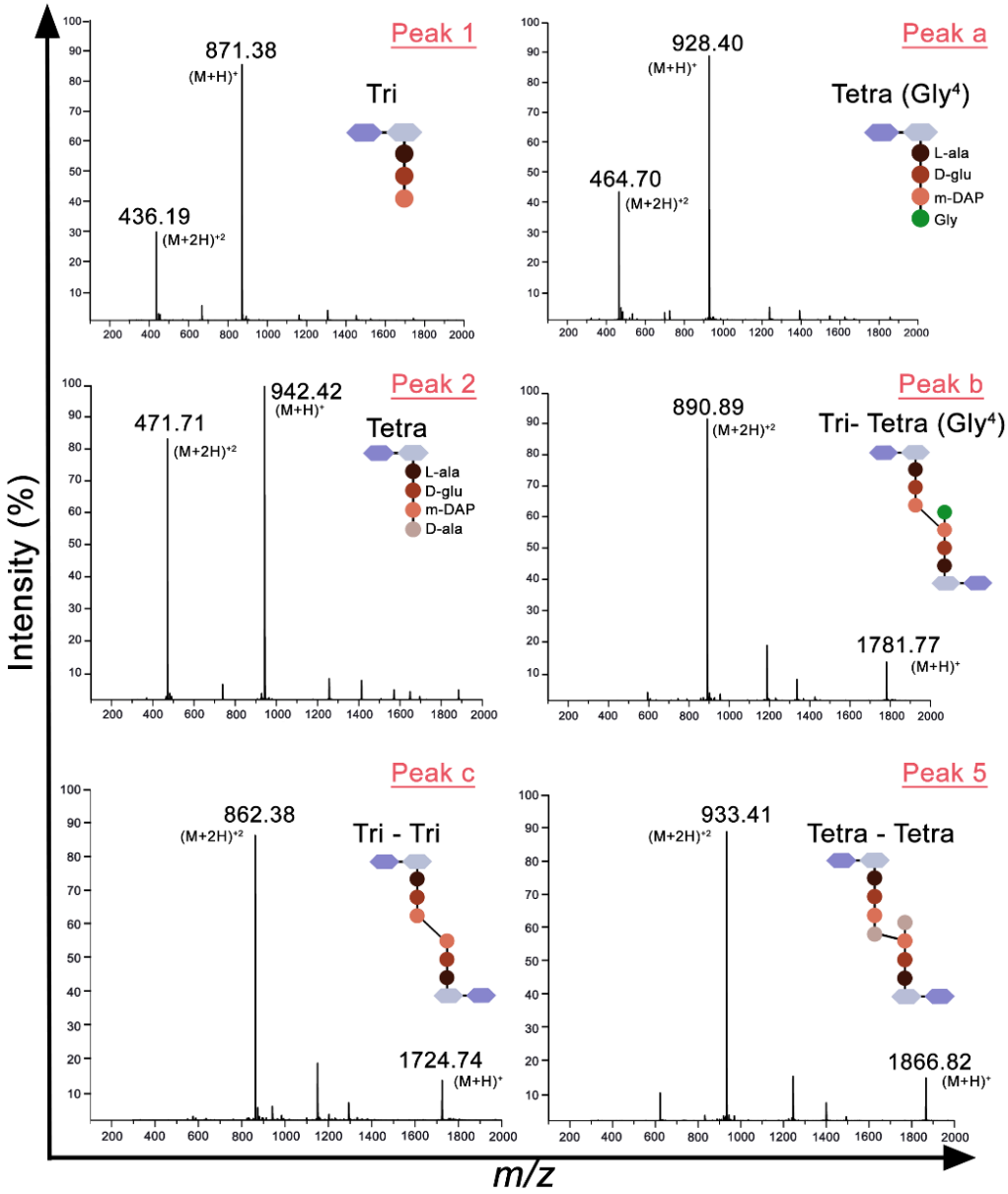
2
3
4
5

Fig. S6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Tri
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D-glu
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Gly
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(M+2H)+2
Tri- Tetra (Gly4)
1781.77
(M+H)+
Peak c
862.38
(M+2H)+2
Tri - Tri
1724.74
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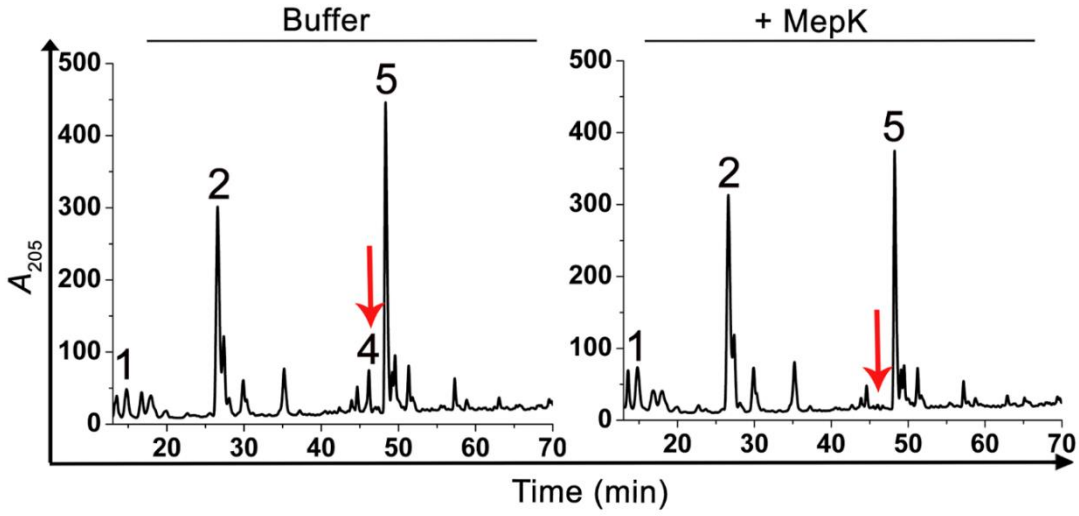
(M+H)+
Intensity (%)
m/z

Fig. S7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Time (min)
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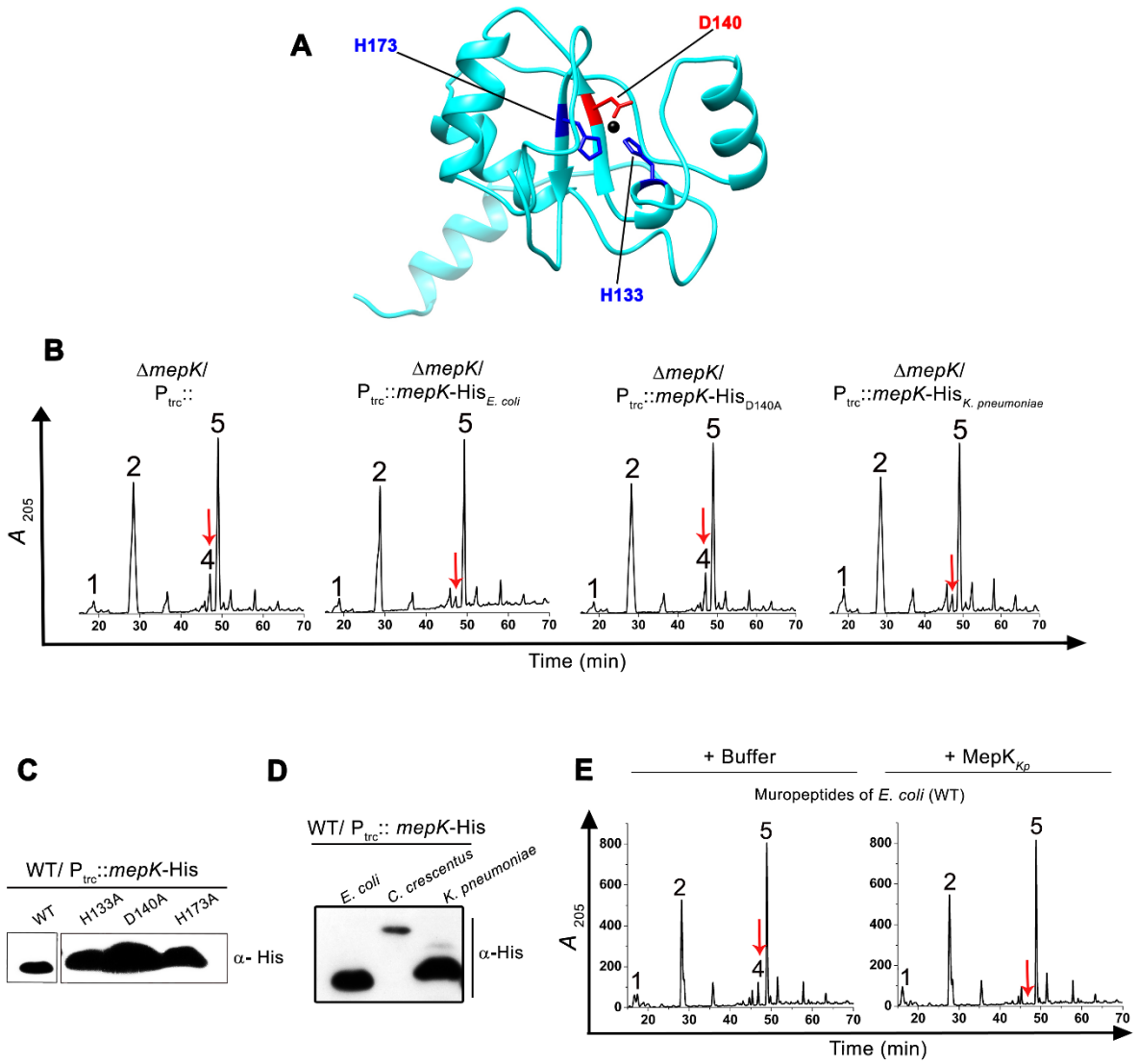
2
4
5

Fig. S8


A
H173
D140
H133
B
ΔmepK/ P trc::
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ΔmepK/ P trc::mepK-His D140A
ΔmepK/ P trc::mepK-His K. pneumoniae
A 205
Time (min)
C
WT/ P trc::mepK-His
WT
H133A
D140A
H173A
α- His
D
WT/ P trc:: mepK-His
E. coli
C. crescentus
K. pneumoniae
α-His
E
+ Buffer
+ MepK Kp
Muropeptides of E. coli (WT)
A 205
Time (min)

Table S1. Strains used in this study

Strains	Genotype[a]	Source/Reference
BW25113	*lacI*^{q} *rrnB*3 Δ*lacZ*4787 Δ(*araBAD*)567 Δ(*rhaBAD*)568 *hsdR*514	[5]
C41 (λDE3)	BL21 (λDE3) with an uncharacterized mutation	[3]
DH5α	F^{-} *hsdR17 deoR recA1 endA1phoA supE44 thi-1 gyrA96 relA1* Δ*(lac-argF)U169* φ*80dlacZ* ΔM15	Lab collection
MG1655	*rph*1 *ilvG rfb*-50	Lab collection
*NA1000	*Caulobacter crescentus*	[Sunish Radhakrishnan]
*KP3384	*Klebsiella pneumoniae*	[Anindya S Ghosh]
JW0909	BW25113 Δ*mepK*::Kan	[5]
JW5204	BW25113 Δ*mepM*::Kan	[5]
JW5580	BW25113 Δ*tatB*::Kan	[5]
JW1668	BW25113 Δ*ldtE*::Kan	[5]
JW2325	BW25113 Δ*mepA*::Kan	[5]
MR506	BW27783 Δ*mepS* Δ*mepM*/ pMN83	[4]
MR510	MG1655 Δ*mepS* Δ*mepM*/ pMN83	[4]
MR810	MG1655 Δ*mepS*::*frt*	[6]
PC001	MG1655 Δ*tatB*::Kan	This study
PC003	MG1655 Δ*mepK*::*frt*	This study
PC004	MR810 Δ*tatB*::Kan	This study
PC006	MR810 Δ*mepK*::Kan	This study

PC038	MR510 Δ*mepK*::Kan	This study
PC107	PC003 Δ*mepA*::Kan	This study
[b]PC189	Δ*ldtE*::*frt ldtD*::Tn*10*dCm	This study
PC190	PC189 Δ*mepS*::Kan	This study
PC191	PC189 Δ*mepK*::Kan	This study
PC192	PC189 Δ*mepK*::*frt* Δ*mepS*::Kan	This study
PC193	MG1655Δ*lysA*::Kan	This study

[a]Deletion alleles used in this study are sourced from Keio collection [5]. The deletion mutations were used after testing for their authenticity (by linkage analysis, PCR and sequence analysis) and introduced into different strain backgrounds by P1 phage-mediated transduction (7).

[b]*ldtD*::Tn*10*dCm was obtained by an insertion of Tn*10*dCm in *ldtD* gene (and behaves like a Δ*ldtD* deletion).

*These strains are kind gifts from Sunish Radhakrishnan, IISER, Trivandrum, India and Anindya Ghosh, IIT, Kharagpur, India.

Table S2. Plasmids used in this study

Plasmid	Relevant Features	Source/Reference
pCA24N	Cm^R, *lacI*^{q}, $P_{T5\text{-}lac}$	[8]
pCA24N-*ldtD*	Cm^R, *lacI*^{q}, $P_{T5\text{-}lac}$::*ldtD*	[8]
pCP20	pSC101(Ts), Amp^R, Cm^R, Flp	[9]
pET21b	ColE1, Amp^R, *lacI*q, T7*lac*	Novagen
pMN83	pBAD33 *mepS*¸ Cm^R	[4]
pTrc99a	ColE1, Amp^R, *lacI*q, P_{trc}	Lab collection
pPK2	pTrc99a-*mepK*	This study
pPK6	pET21b-*mepK*$^{31\text{-}182}_{E.\ coli}$	This study
pPK17	pTrc99a-*mepK*-$His_{E.\ coli}$	This study
pPK19	pTrc99a *mepK*-His_{H133A}	This study
pPK20	pTrc99a *mepK*-His_{D140A}	This study
pPK21	pTrc99a *mepK*-His_{H173A}	This study
pPK23	pTrc99a *mepK*-$His_{C.\ crescentus}$	This study
pPK25	pTrc99a *mepK*-$His_{K.\ pneumoniae}$	This study
pPK29	pET21b- *mepK*$^{32\text{-}183}_{K.\ pneumoniae}$	This study

Table S3. Muropeptide composition of various strains

Muropeptide (Peak)	% Area of muropeptide peaks[a]			
	WT	Δ*mepK*	WT/P_{trc}::*mepK*	*mepK*/P_{trc}::*mepK*
Tri (1)	5.56 ± 1.17	4.45 ± 0.03	6.97 ± 0.51	5.92 ± 0.48
Tetra (2)	36.53 ± 1.65	33.89 ± 3.94	39.02 ± 1.83	39.88 ± 2.5
Tri-Tetra (4)	**4.16 ± 0.62**	**7.0 ± 0.43**	**2.05 ± 0.4**	**2.19 ± 0.18**
Tetra-Tetra (5)	28.52 ± 0.96	28.90 ± 1.53	30.94 ± 3.16	32.75 ± 0.43

[a]Muropeptide analysis was done by calculating the relative percentage area of each muropeptide from the HPLC chromatograms (4). Values are derived from three independent experiments and are shown along with standard deviation. Tri-Tetra muropeptide (peak 4) values are shown in bold.

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