APPENDIX

Role of endopeptidases in peptidoglycan synthesis mediated by alternative cross-linking enzymes in *Escherichia coli*

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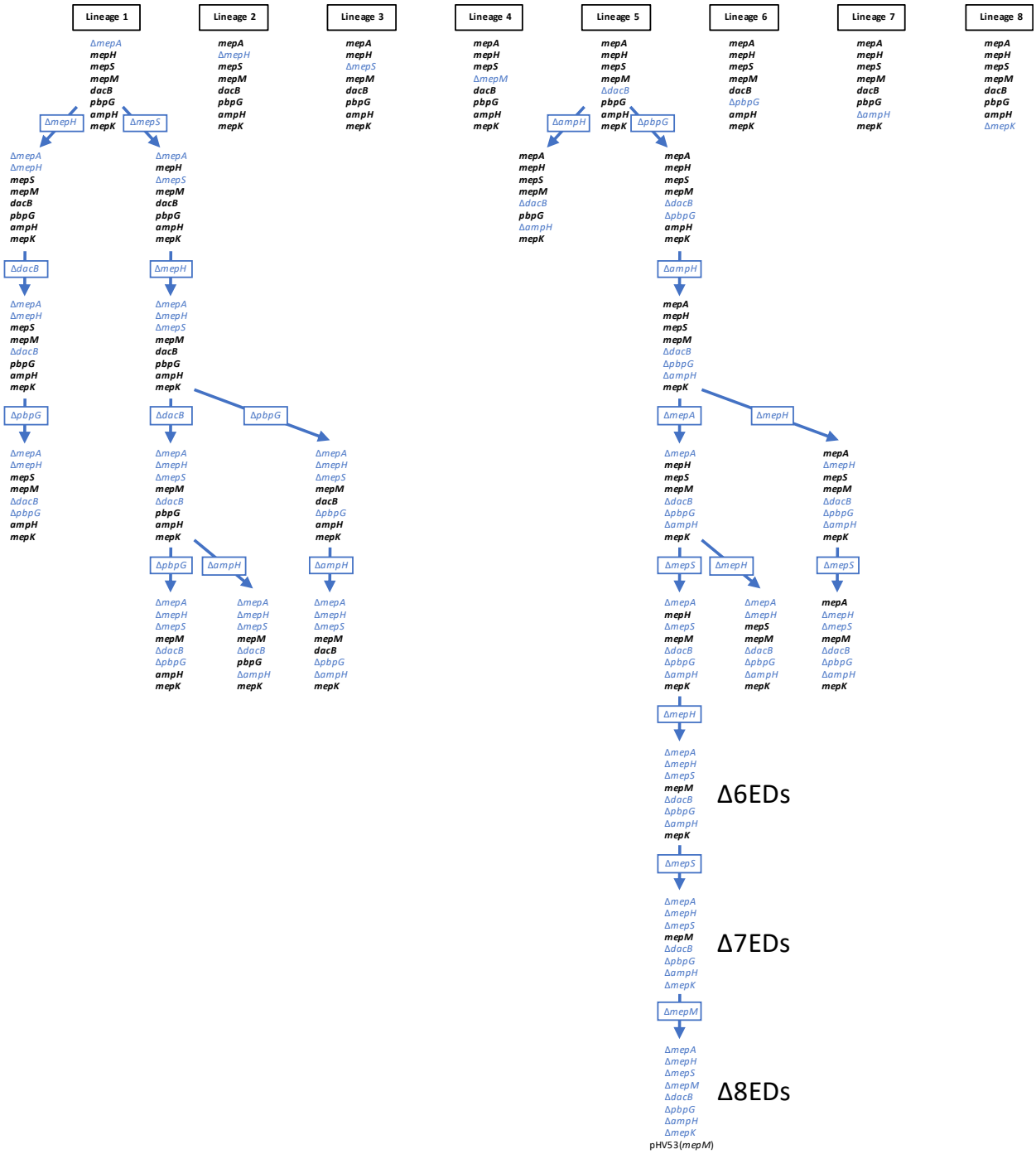

Appendix Figure S1. Parallel and serial deletion of endopeptidase genes in *E. coli* BW25113 Δ*relA*. Deletions indicated in blue were introduced by the procedure of Datsenko and Wanner (Datsenko & Wanner, 2000). The remaining endopeptidase genes are indicated in black. The presence of all deletions was verified by PCR at each steps. The genomes of the strains retaining *mepM* and *mepK* (Δ6EDs) or only *mepM* (Δ7EDs) were re-sequenced and no compensatory mutation was detected.

Table S1. Characteristics and origin of the plasmids used in this study

Plasmid	Characteristics				Origin
Vectors					
pHV6	Tet^R	P_{trc}	*lacI*	*oriV* CloDF13	This study
pHV7	Cm^R	P_{araBAD}	*araC*	*oriV* P15a	This study
pHV9	Zeo^R	P_{phlF}	*phlF*	*oriV* pBR322	This study
pHV30	Zeo^R	P_{rhaBAD} TIS1	*rhaSR*	*oriV* pSC101	This study
pET-TEV	Km^R	P_{T7}	*lacI*	*oriV* ColE1	(Houben et al., 2007)
pETMM82	Km^R	P_{T7} *dsbC*	*lacI*	*oriV* ColE1	(Firczuk & Bochtler, 2007)
Recombinant plasmids for ycbB and relA' expression					
pKT2	pHV6Ω*ycbB*				(Hugonnet et al., 2016)
pKT8	pHV7Ω*relA'*				(Hugonnet et al., 2016)
pHV63	pHV7Ω*ycbB*				This study
Recombinant plasmids for complementation of endopeptidase gene deletions					
pHV10.1	pHV9Ω*mepM*				This study
pHV10.2	pHV9Ω*mepM* $H^{393}A$				This study
pHV10.3	pHV9Ω*mepM*Δ936-1224 (ΔLytM domain)				This study
pHV43.1	pHV30Ω*mepA*				This study
pHV43.2	pHV30Ω*mepA* $H^{113}A$				This study
pHV44	pHV30Ω*mepH*				This study
pHV45.1	pHV30Ω*mepK*				This study
pHV45.2	pHV30Ω*mepK* $H^{133}A$				This study
pHV46	pHV30Ω*mepM*				This study
pHV47.1	pHV30Ω*mepS*				This study
pHV47.2	pHV30Ω*mepS* $C^{94}A$				This study
pHV48	pHV30Ω*dacB*				This study
pHV49	pHV30Ω*pbpG*				This study
pHV50	pHV30Ω*ampH*				This study
pHV53	pHV30ΩTIS2-*mepM*				This study
pHV55	pHV7Ω*mepA*				This study
pHV56	pHV7Ω*mepH*				This study
pHV57	pHV7Ω*mepK*				This study
pHV58	pHV7Ω*mepM*				This study
pHV59	pHV7Ω*mepS*				This study
pHV60	pHV7Ω*dacB*				This study
pHV61	pHV7Ω*pbpG*				This study
pHV62	pHV7Ω*ampH*				This study
Recombinant plasmids for protein production					
pET-TEVΩ*mepM*Δ1-120	Production of MepM Δ1-40				This study
pETMM82Ω*mepH*Δ1-81	Production of MepH Δ1-27				This study
pETMM82Ω*mepS*Δ1-72	Production of MepS Δ1-24				This study
pET21bΩ*dacB*Δ1-60	Production of PBP4 Δ1-20				(Banzhaf et al., 2020)
pET28aΩ*pbpG*Δ1-75	Production of PBP7 Δ1-25				(Banzhaf et al., 2020)

Table S2. Characteristics and origin of *E. coli* strains used in this study

Strain	Characteristics	Origin
BW25113	Δ(*araD-araB*)*567* Δ(*rhaD-rhaB*)*568* Δ*lacZ4787* (::rrnB-3) *hsdR514 rph-1*	(Baba et al., 2006)
BW25113(*ycbB, relA'*)	Δ*relA* derivative of BW25113 harboring pKT2(*ycbB*) and pKT8(*relA'*)	(Hugonnet et al., 2016)
BW25113 M1	Ampicillin resistant derivative of BW25113 pJEH12(*ycbB*) harboring a mutation in the 5' UTR region of *ileRS*	(Hugonnet et al., 2016)
BL21(DE3)	Host for protein production	(Wood, 1966)

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