Supplemental Information to

Listeria monocytogenes genes supporting growth under standard laboratory cultivation conditions and during macrophage infection

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Supplemental Table S1: Genes required for *L. monocytogenes* growth (please see separate excel file).
Supplemental Table S2: Genes with high PMS frequency in the *L. monocytogenes* population.
Supplemental Table S3: Plasmids and strains used in this study.
Supplemental Table S4: Oligonucleotides used in this study.

Supplemental Figure S1: Tn insertion maps of *L. monocytogenes* genes (potentially) required for growth (please see separate pdf file).
Supplemental Figure S2: Selected characteristics of *L. monocytogenes* genes (potentially) required for growth but with unknown function.
Supplemental Figure S3: Distribution of Tn insertions in selected genes categorized as required for growth in this study but that could be deleted in previous studies.
Supplemental Figure S4: Necessity of envelope biosynthesis genes for growth of *L. monocytogenes*.
Supplemental Figure S5: Necessity of genes acting in biosynthesis of selected cofactors for *L. monocytogenes* growth.
Supplemental Figure S6: Necessity of genes for heme, cobalamin and menaquinone biosynthesis for *L. monocytogenes* growth.
Supplemental Figure S7: Growth of a *L. monocytogenes* mutant lacking *whiA* (*lmo2472*).

Supplemental File S1: In-house script for conversion of SAM to wig files (please see separate txt file).

Supplemental Table S2: Genes with high PMS frequency in the *L. monocytogenes* population

locus tag	gene symbol	product	required for growth	PMS frequency (%)
lmo2760a		hypothetical protein	no	56.41
lmo0380		hypothetical protein	no	22.71
lmo0071		hypothetical protein	no	8.85
lmo1124		hypothetical protein	no	5.61
lmo0671		hypothetical protein, secreted	no	5.29
lmo0175		peptidoglycan-binding protein, LPXTG motif,	no	3.91
lmo0524		sulfate transporter	no	3.84
lmo1638		putative muramoyltetrapeptide carboxypeptidase	no	3.60
lmo0433	*inlA*	internalin A	no	3.58
lmo2724		putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein	yes	3.55
lmo2290		protein gp13	no	2.64
lmo1412	*flaR*	modulates DNA topology	no	2.56
lmo0117	*lmaB*	antigen B	no	2.56
lmo1076	*auto*	autolysin	no	2.53
lmo2809		hypothetical protein	no	2.43
lmo0148		hypothetical protein	no	2.34
lmo0586		hypothetical protein, CscA-like	no	2.14
lmo2733		PTS fructose transporter subunit IIABC	no	1.82
lmo1117		hypothetical protein	no	1.69
lmo0859		sugar ABC transporter substrate-binding protein	no	1.62
lmo0940		hypothetical protein	no	1.60
lmo2771		beta-glucosidase	no	1.35
lmo0395		blasticidin S-acetyltransferase	no	1.31
lmo1308		hypothetical protein	no	1.29
lmo0401		alpha-mannosidase	no	1.24
lmo2375		hypothetical protein	no	1.05
lmo2781		beta-glucosidase	no	1.05
lmo0660		transposase	no	1.00

Supplemental Table S3: Plasmids and strains used in this study

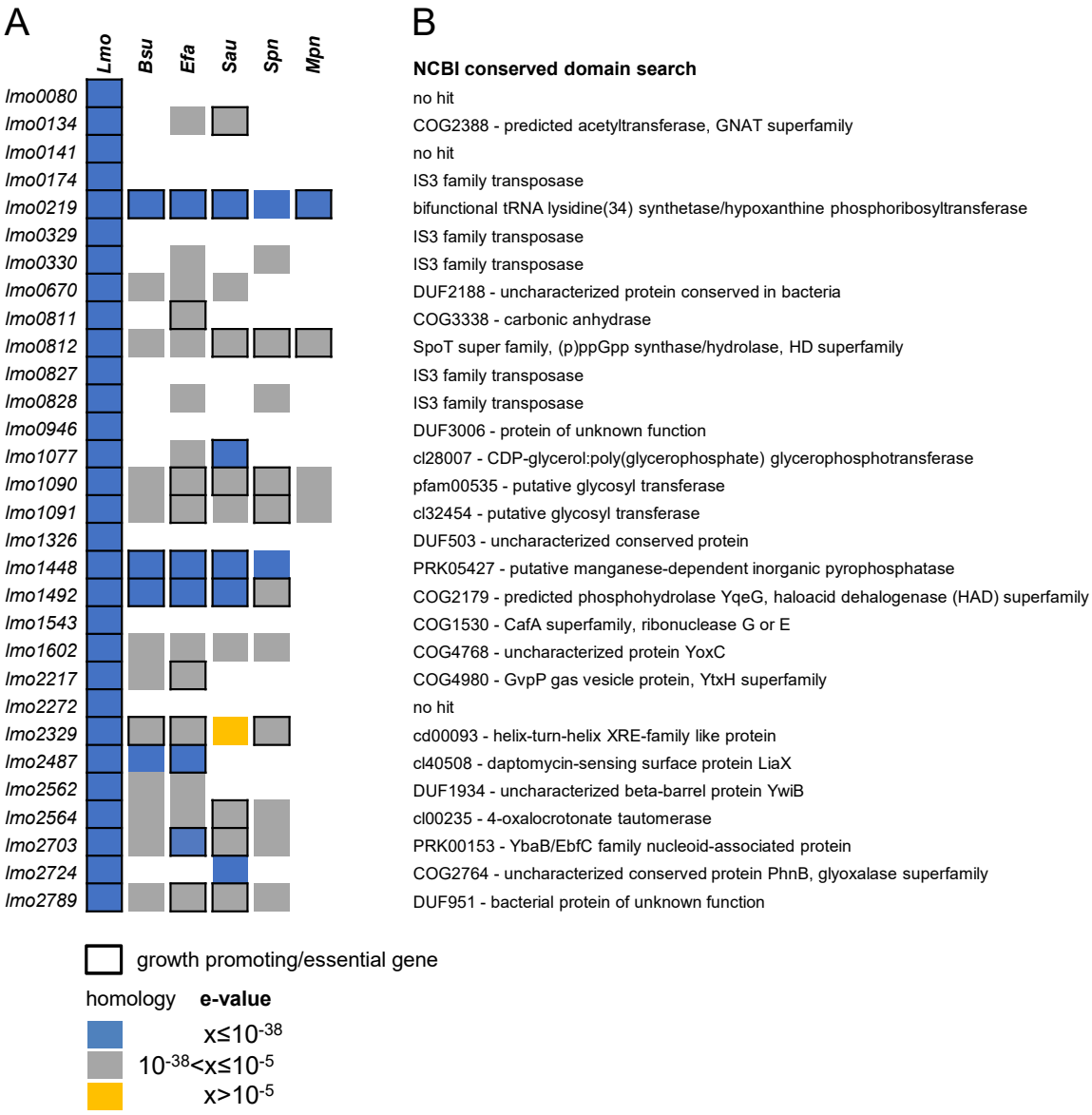
name	relevant characteristics	source*/ reference
plasmids		
pKRMIT	transposon delivery vector	(Le Breton et al. 2015)
pMAD	*bla erm bgaB*	(Arnaud et al. 2004)
pJR57	*bla erm bgaB* Δ*cozEb* (*lmo0584*)	this work
pSH541	*bla erm bgaB* Δ*hly* (*lmo0202*)	this work
pSH542	*bla erm bgaB* Δ*actA* (*lmo0204*)	this work
pSH553	*bla erm bgaB whiA* (*lmo2472*) region	this work
pSH554	*bla erm bgaB prkA*ΔC (*lmo1820*)	this work
pSH556	*bla erm bgaB* Δ*whiA* (*lmo2472*)	this work
pSH559	*bla erm bgaB* Δ*walJ* (*lmo0291*)	this work
pSH563	*bla erm bgaB* Δ*ftsK* (*lmo1386*)	this work
pSH566	*bla erm bgaB* Δ*parA* (*lmo2791*)	this work
pTE11	*bla erm bgaB* Δ*lmo2214-lmo2215*	this work
pTE51	*bla erm bgaB* Δ*thrC* (*lmo2546*)	this work
pTE57	*bla erm bgaB* Δ*gcvPAB* (*lmo1349-lmo1350*)	this work
***L. monocytogenes* strains**		
EGD-e	wild-type, serovar 1/2a strain	(Glaser et al. 2001)
BUG2214	Δ*prfA*	(Mandin et al. 2007)
LMJR138	Δ*clpC*	(Rismondo et al. 2017)
LMS2	Δ*divIVA*	(Halbedel et al. 2012)
LMS81	Δ*secA2*	(Halbedel et al. 2012)
LMSW84	Δ*prkA attB::*P_{help}-*lacO-prkA lacI neo*	(Wamp et al. 2020)
LMJR87	Δ*cozEb*	pJR57 ↔ EGD-e
LMS250	Δ*hly*	pSH541 ↔ EGD-e
LMS251	Δ*actA*	pSH542 ↔ EGD-e
LMS278	*prkA*ΔC	pSH554 ↔ EGD-e
LMS281	Δ*whiA*	pSH556 ↔ EGD-e
LMS283	Δ*walJ*	pSH559 ↔ EGD-e
LMS284	Δ*ftsK*	pSH563 ↔ EGD-e
LMS290	Δ*parA*	pSH566 ↔ EGD-e
LMS305	Δ*gcvPAB*	pTE57 ↔ EGD-e
LMTE009	Δ*lmo2214-lmo2215*	pTE11 ↔ EGD-e
LMTE36	Δ*thrC*	pTE51 ↔ EGD-e

* The double arrow (↔) indicates gene deletions obtained by chromosomal insertion and subsequent excision of pMAD plasmid derivatives (see experimental procedures for details).

Supplemental Table S4: Oligonucleotides used in this study.

name	sequence (5′→3′)*
JR153	GCGCGGATCCCGTCTAGAAGGAGCAAACTACGATATTCG
JR154	GCGCGGAATTCCCAGCTCCAGTTCCACGACGGTCAG
KK103	GTGGGATCCTAAAATAAAAAACACTATTAGTTAGAAAAT
KK104	TTAGGATCCCACTTTACCACCCTCTTTTG
MF38	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**NN
MF52	**CTGTCTCTTATACACATCTCCGAGCCCACGAGAC**
MF53	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGAGGAATTTGTATCG
MF93	GATCTATCGATGCATGCCATGGATGTGCTTCCACCAGGTGAT
MF94	CGCGTCGGGCGATATCGGATCCCCCGCGACGAATTTCATCTG
MF95	CCTTTTCTTTTTAAGGCTTACTCACTCCTTTTCATTAGTTTGG
MF96	GAGTGAGTAAGCCTTAAAAAGAAAAGGATGCGAATTAGAATGTC
SHW838	CAGATCTATCGATGCATGCCATGGGAATTGCAATAATGCCTTTTGGGATG
SHW839	GATATCGGATCCATATGACGTCGACCCACTGTATCCTTTTTCCATTCAG
SHW840	TATAAGTGGGATCCTAAAAACACAGAACGAAAGAAAAAGTG
SHW841	TGTTTTTAGGATCCCACTTATACTCCCTCCTCGTGATAC
SHW858	CGTTACACATTAACTAGACAGATCTGCTCGTGTCAGTTCTGGGAGTAG
SHW859	CGGATCCATATGACGTCGACGAGCTTCACTCTGACCGATGGC
SHW860	CAATTACTGCAGCATGGGTTTCACTCTCCTTCTAC
SHW861	CCCATGCTGCAGTAATTGTAAAAGTAATAAAAAATTAAGAA
SHW899	CGTTACACATTAACTAGACAGATCTTTGTAATGGAACATGTAGATGGG
SHW900	CGCGTCGGGCGATATCGGATCCTTCGTTTTCGTGCATACAGCC
SHW901	ATTTTACTGCAGTAAAAATAGAACGGAGGAAATGTGC
SHW902	CTATTTTTACTGCAGTAAAATACCGATGATAAAAATGATAATG
SHW911	GATCTATCGATGCATGCCATGGCTGGTAAGCAAATTCTTCCCG
SHW912	GCGTCGGGCGATATCGGATCCCGCGACGTTTAATCGAAACTGCCG
SHW913	GGTGAAAAAGAATGGCTAAAGGTCTAGGTAAAGG
SHW914	CCTTTAGCCATTCTTTTTCACCAACCCTTATTTTG
SHW915	CTAGACAGATCTATCGATGCATGCCATGGCCATTTTTCATCATAGGTTACCG
SHW916	GGGCGATATCGGATCCATATGACGTCGACAAACTGAGGTTGCAACTCCCG
SHW917	TTTTTTTAAAGCTTCATTTCAAGTCCCACCCATTCC
SHW918	TTGAAATGAAGCTTTAAAAAAAGGTTGCGATTTTTAATCGC
SHW919	CAGATCTATCGATGCATGCCATGGCTTCACTACAGAGCGAGCAGC
SHW920	CGCGTCGGGCGATATCGGATCCCGATGTAGGCTTTTCCATTTGCC
SHW921	AAAAAATGGTCGACTAAGTTTCACCGTTTTTTTAAAGC
SHW922	GAAACTTAGTCGACCATTTTTTATCCCCCTAAAGGC
SHW942	CAGATCTATCGATGCATGCCATGGTATATGCCAAGCGCAGATGCAGG
SHW943	CGCGTCGGGCGATATCGGATCCCCTTCAAAGTGATTGATGGTGGC
SHW944	TTTTTAGTCGACCATATTACTTCCTCCTTTTTACTTTGC
SHW945	AATATGGTCGACTAAAAAGAAGCCTTGCTGCGAAATTTC
TE7	GGAGGTATGCTGCAGTGAAAAAAGCGATTTGGATGATTG
TE8	GCTTTTTTCACTGCAGCATACCTCCTTACGTAATCTTTAG
TE84	CGATGCATGCCATGGTACCCGGGCTCACAAAAGCGATGAATATG
TE85	GATATCGGATCCATATGACGTCGACCTTGTGTGTACCAGTCTGCG
TE202	GGCGATATCGGATCCATATGACGTCGACCCCCTACGACCCAGTTTCCAAG
TE203	GATCTATCGATGCATGCCATGGTACCCGGGGTGCAGCTGTCGGAGAAACAATG
TE204	CACGAATACGCATTACTGCAGCATATTTAACCCTCCACAACGG
TE205	GAGGGTTAAATATGCTGCAGTAATGCGTATTCGTGTCCCGGC

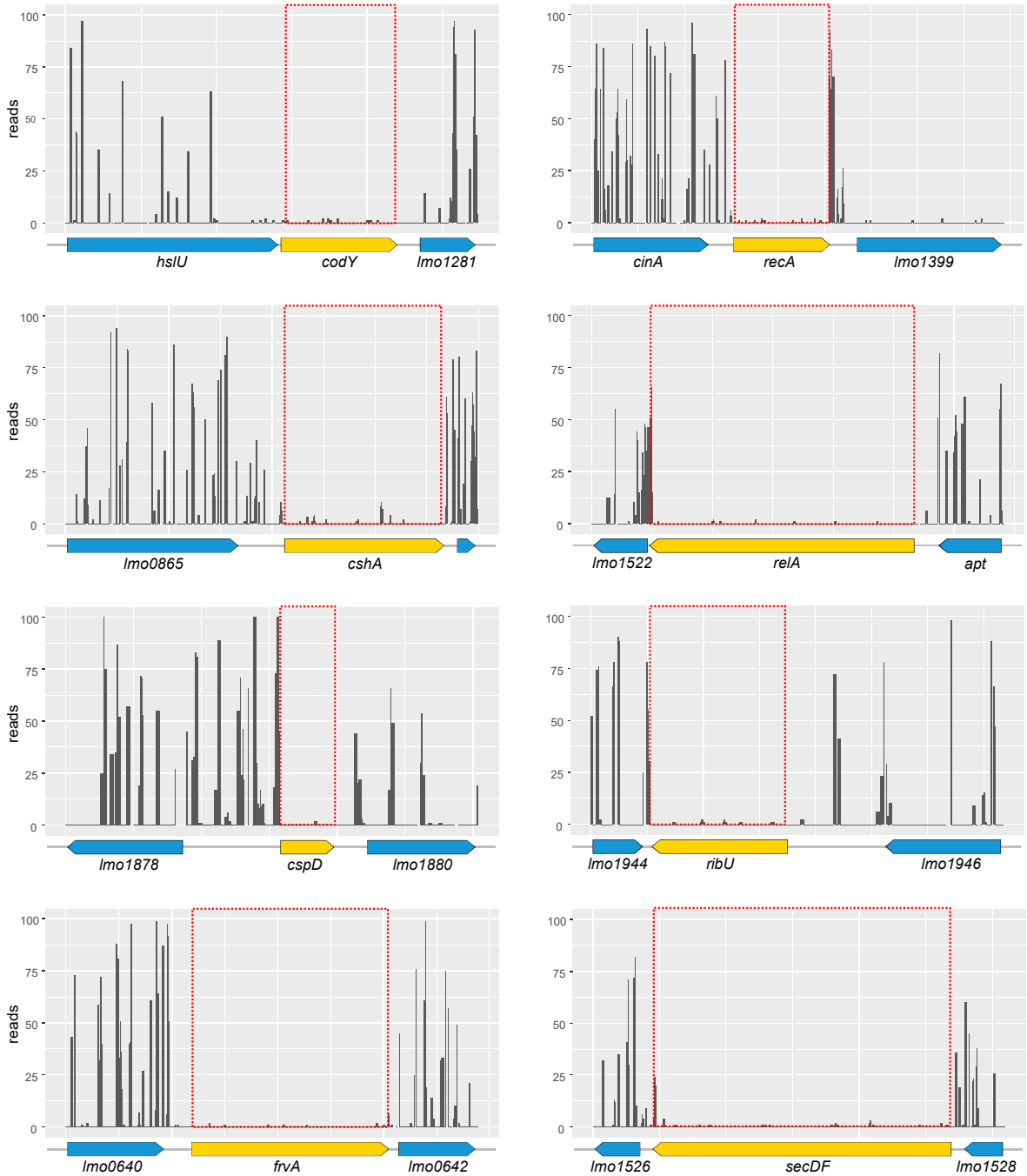
* Illumina adapter sequences included in the primers are shown in bold. Complementary sequence of MF38 and MF52 necessary for the formation of double stranded DNA in the ligation step are underlined.



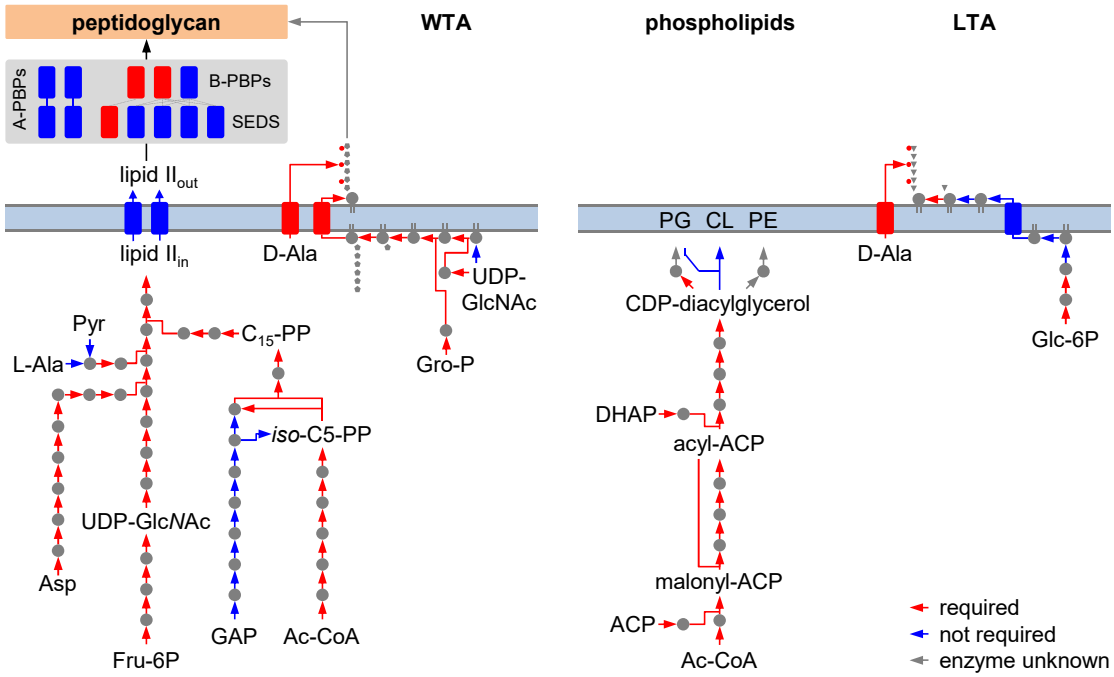
Supplemental Figure S2: Selected characteristics of *L. monocytogenes* genes (potentially) required for growth but with unknown function.

(A) Presence and essentiality of homologs in selected Gram-positive species (*Lmo* – *L. monocytogenes*, *Bsu* – *Bacillus subtilis*, *Efa* – *Enterococcus faecalis*, *Sau* – *Staphylococcus aureus*, *Spn* – *Streptococcus pneumoniae*, *Mpn* – *Mycoplasma pneumoniae*). Coloring indicates degree of sequence similarity (e-value). Black contours indicate necessity for growth/essentiality in the particular species (*Lmo*: this work; *Bsu*: Commichau *et al.*, 2013, Kobayashi *et al.*, 2003; *Efa*: Gilmore *et al.*, 2020; *Sau*: Valentino *et al.* 2014; *Spn*: Le Breton *et al*., 2015; *Mpn*: Lluch-Senar *et al.*, 2015).

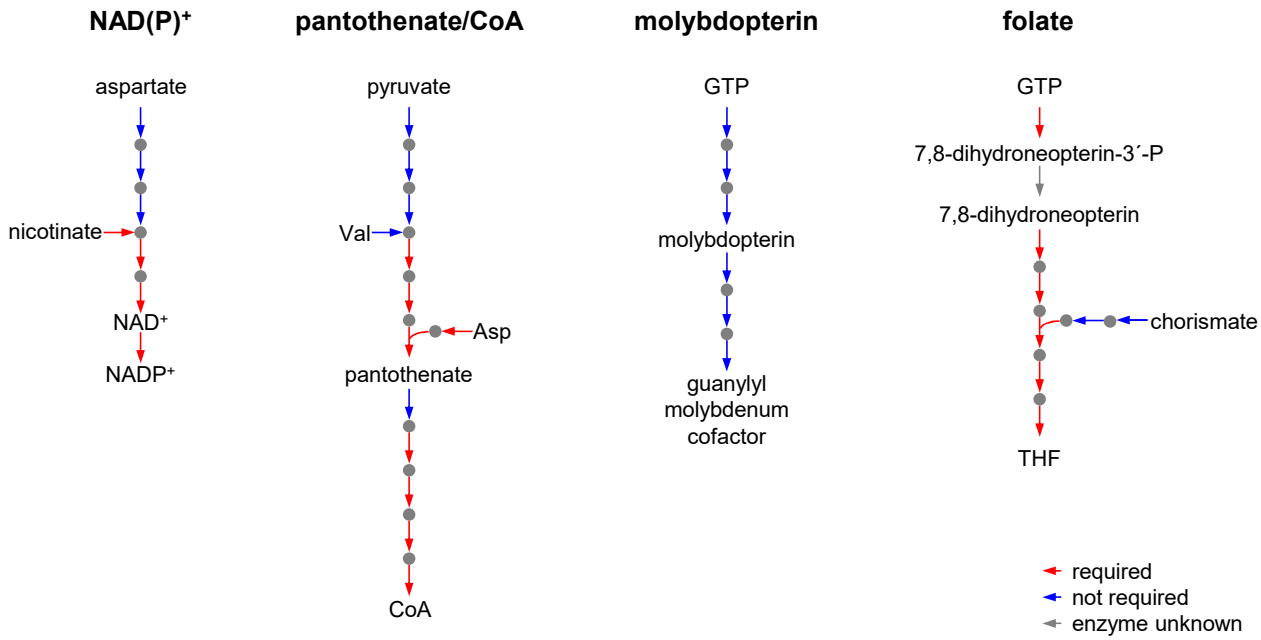
(B) Results of an conserved domain search using the NCBI conserved domain search web portal.



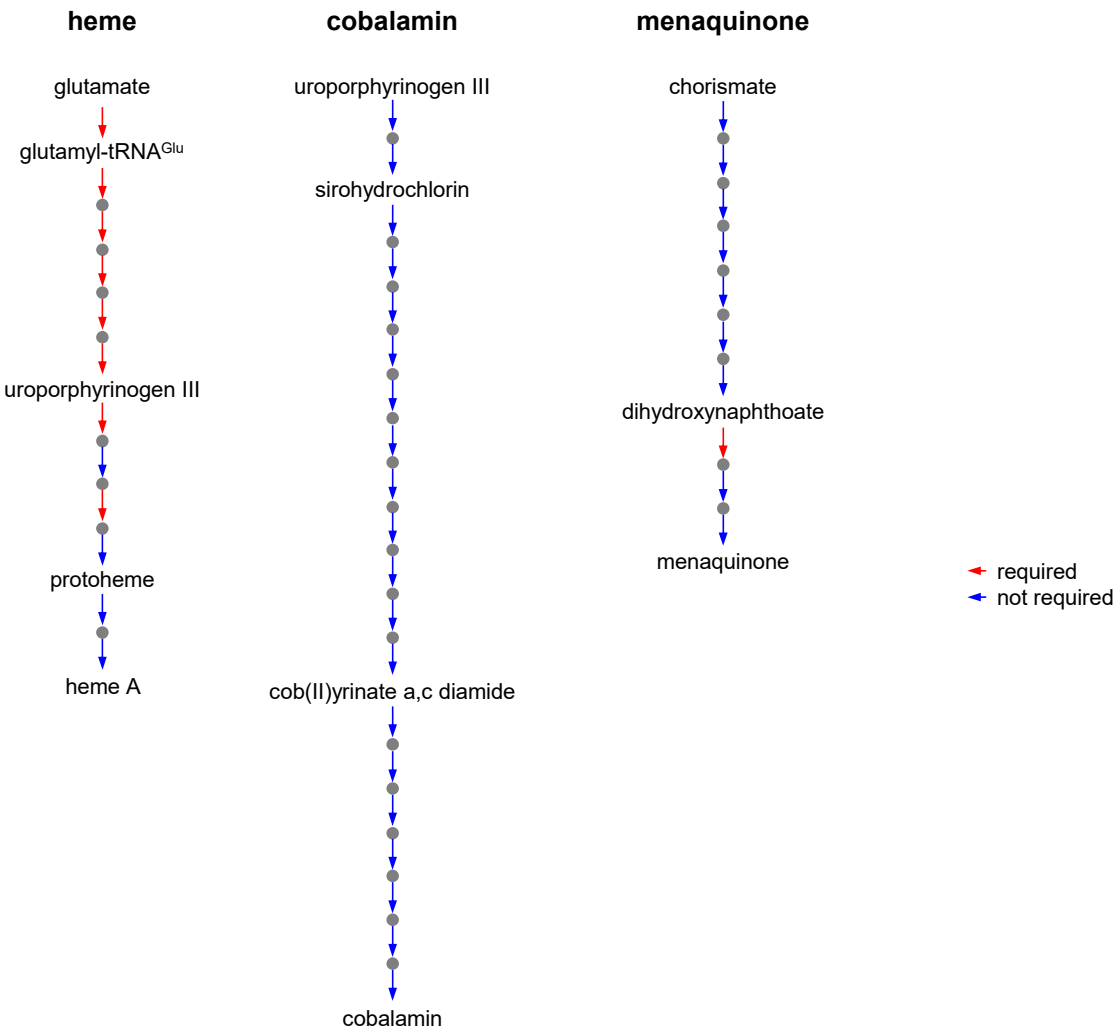
Supplemental Figure S3: Distribution of Tn insertions in selected genes categorized as required for growth in this study but that could be deleted in previous studies.
Distribution of Tn insertions at the *codY*, *cshA*, *cspD*, *frvA*, *recA*, *relA, ribU* and *secDF* loci of Tn mutagenized *L. monocytogenes* EDG-e, which resulted in their categorization as genes required for growth during standard cultivation conditions in this study.



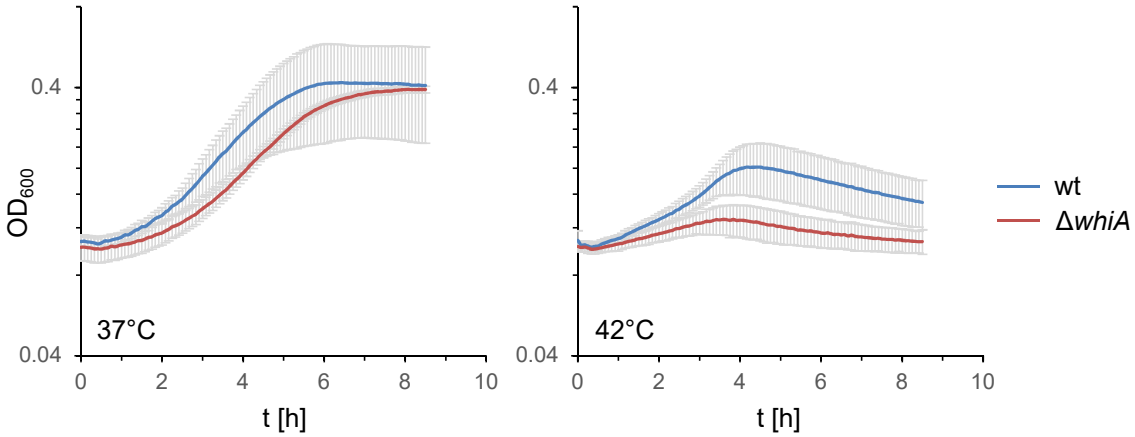
Supplemental Figure S4: Necessity of envelope biosynthesis genes for growth of *L. monocytogenes*.
Schemes illustrating the *L. monocytogenes* pathways for biosynthesis of peptidoglycan and wall teichoic acids (WTA, left), phospholipids (middle) and lipoteichoic acids (LTA, right) according to the KEGG database (https://www.genome.jp/kegg-bin/show_organism?org=T00066). Reactions are colored according to the requirement of their corresponding genes for growth under standard cultivation conditions. Pathways for LTA and WTA glycosylation were not included.



Supplemental Figure S5: Necessity of genes acting in biosynthesis of selected cofactors for *L. monocytogenes* growth.
Schemes illustrating the anabolic pathways for biosynthesis of NAD(P)+, coenzyme A, molybdopterin and folate according to the KEGG database (https://www.genome.jp/kegg-bin/show_organism?org=T00066). Reactions are colored according to the requirement of their corresponding genes for growth under standard cultivation conditions.



Supplemental Figure S6: Necessity of genes for heme, cobalamin and menaquinone biosynthesis for *L. monocytogenes* growth.
Schemes illustrating the anabolic pathways for heme, cobalamin and menaquinone biosynthesis according to the KEGG database (https://www.genome.jp/kegg-bin/show_organism?org=T00066). Reactions are colored according to the requirement of their corresponding genes for *L. monocytogenes* growth under standard cultivation conditions.



Supplemental Figure S7: Growth of a *L. monocytogenes* mutant lacking *whiA* (*lmo2472*).
Growth of *L. monocytogenes* strains EGD-e (wt) and LMS281 (Δ*whiA*) in BHI broth in a plate reader at 37°C and 42°C. The experiment was performed with three replicates. Average values and standard deviations are shown.

Supplemental Information – References

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