

Table S1. Strains, plasmids, and PCR primers

Strain, primer, or plasmid	Relevant features (Primer sequence 5'-3')	Source or reference
Strains		
<i>L. monocytogenes</i> EGDe	Serotype 1/2a strain; genome is sequenced	(1)
<i>E. coli</i> EC1000	Cloning host strain	Invitrogen
$\Delta yqgS$	EGDe $\Delta yqgS$	This study
$\Delta lafA$	EGDe $\Delta lafA$	This study
$\Delta virR$	EGDe $\Delta virR$	Lab collection
$\Delta gtcA$	EGDe $\Delta gtcA$	This study
$\Delta lmo1464$	EGDe $\Delta lmo1464$	This study
Plasmids		
pGPA2	mariner delivery vector; Cm ^r ; Gm ^r	(2)
pWS3	Temperature-sensitive integration vector; Spc ^r	(3)
pWS3-erm-cre	Temperature-sensitive integration vector; Ery ^r	This study
Primers		
pAT392_lox66_genta_F	TACCGTTCGTATAGCATAACATTATACGA AGTTATGATAAACCCAGCGAACCATTT GAGG	(3)
pAT392_lox71_genta_R	TACCGTTCGTATAATGTATGCTATACGA AGTTATTCAATCTTTATAAGTCCTTTTAT AA	(3)
genta2_F	ACCTCAAGTACCGTTCGTATAGCATAACA	This study
genta2_R	GTGTAGCTTACCGTTCGTATAATGTATG	This study
Genta2_1_R	TCAAGGCAATCTGCCTCCTCA	This study
Genta2_2_F	ATGGAAAGACTAAATGCAACAACA	This study
pWS3_gm_ud_F1	GGGGATTTTATGCGTGAGAA	This study
pWS3_gm_ud_R1	GCTTCCAAGGAGCTAAAGAGG	This study
<i>yqgS</i> _up_F	AAGCTGAATTCCTGCAGCCCCGGGGAT CAAAGTCATCGCA	This study
<i>yqgS</i> _up_R	TGTATGCTATACGAACGGTACTTGAGGT ACGCTTTGACGCTAGCCGAA	This study
<i>yqgS</i> _down_F	GCATACATTATACGAACGGTAAGCTACA CAGCAGGTCCATTCCAAGTGC	This study
<i>yqgS</i> _down_R	CTAGAACTAGTGGATCCCCCTCGCTGC AATCACGAAGGAA	This study
Check_ <i>yqgS</i> _F	GAACCAGCTGAAGGTACGGT	This study
Check_ <i>yqgS</i> _R	ACTGCAAGCCCGTATTCCAA	This study
<i>lafA</i> _up_F	AAGCTGAATTCCTGCAGCCCCGAGCA AGTTCCTTCTCTGT	This study

<i>lafA</i> _up_R	TGTATGCTATACGAACGGTACTTGAGGT ACGTTCAAAGGAAAGAGAGGTC	This study
<i>lafA</i> _down_F	GCATACATTATACGAACGGTAAGCTACA CTGCGGACTGTAGGTATCCGT	This study
<i>lafA</i> _down_R	CTAGA ACTAGTGGATCCCCAAGTAAT CGGCCCTGGTGTG	This study
Check_ <i>lafA</i> _F	GAAAATCCGCCTGCCCAAAC	This study
Check_ <i>lafA</i> _R	CCGAACTTACAGGTGCTCCA	This study
<i>gtcA</i> _up_F	AAGCTGAATTCCTGCAGCCCCCAGCAA AAGCTGAGTGCG	
<i>gtcA</i> _up_R	TGTATGCTATACGAACGGTACTTGAGGT ATGGGCAAAAATTTGGACGAA	This study
<i>gtcA</i> _down_F	GCATACATTATACGAACGGTAAGCTACA CACCACGGAATCTTGTCTAACCAT	This study
<i>gtcA</i> _down_R	CTAGA ACTAGTGGATCCCCAAACCCC GGTTCTTCCAGAT	This study
Check_ <i>gtcA</i> _F	TGCTTCCACTTGAAAACCTCCT	This study
Check_ <i>gtcA</i> _R	ACAACATCCGCCAGAGCTAA	This study
<i>lmo1464</i> _up_F	AAGCTGAATTCCTGCAGCCCACCGTCG CTGTTTTTCCTGT	This study
<i>lmo1464</i> _up_R	TGTATGCTATACGAACGGTACTTGAGGT AGCGGTACTGTTAGCAGCAAT	This study
<i>lmo1464</i> _down_F	GCATACATTATACGAACGGTAAGCTACA CAAAAGCATGATGAAAAGATTCTGC	This study
<i>lmo1464</i> _down_R	CTAGA ACTAGTGGATCCCCCAGGAGGG CAAAAGATGACGG	This study
Check_ <i>lmo1464</i> _F	TGTGAATGCGCCTGGTAAAA	This study
Check_ <i>lmo1464</i> _R	TCTTCCACAGACCAACCAT	This study

Table S2. *L. monocytogenes* genes involved in nisin resistance were determined by Tn-seq analysis.

ORF	Gene	Product	Fold	BH
Cell wall envelope				
<i>lmo2477</i>	<i>galE</i>	UDP-glucose 4-epimerase	5851.31	0.00E+00
<i>lmo2554</i>	<i>lafB</i>	galactosyltransferase	521.46	3.78E-10
<i>lmo0644</i>	<i>yqgS</i>	glycolipid synthesis protein	343.00	2.10E-10
<i>lmo2555</i>	<i>lafA</i>	N-acetylglucosaminyl-phosphatidylinositol biosynthesis protein	135.98	2.05E-09
<i>lmo1695</i>	<i>mprF</i>	hypothetical protein	10.92	2.13E-06
<i>lmo2229</i>	<i>pbp2</i>	penicillin-binding protein	6.69	8.51E-05
<i>lmo0974</i>	<i>dltA</i>	D-alanine--poly(phosphoribitol) ligase subunit 1	6.64	2.45E-02

<i>lmo2553</i>	<i>lafC</i>	hypothetical protein	6.54	2.70E-05
<i>lmo0855</i>	<i>ddl</i>	D-alanyl-alanine synthetase A	6.43	9.01E-05
<i>lmo2505</i>	<i>spl</i>	peptidoglycan lytic protein P45	6.18	2.66E-04
<i>lmo2427</i>	<i>ftsW</i>	cell division protein FtsW	5.31	1.04E-05
regulator				
<i>lmo1745</i>	<i>virR</i>	two-component response regulator	121.05	8.34E-09
<i>lmo1022</i>	<i>liaR</i>	two-component response regulator	77.68	1.61E-06
<i>lmo0989</i>		MarR family transcriptional regulator	44.69	1.22E-03
<i>lmo1693</i>	<i>recX</i>	recombination regulator RecX	22.93	9.51E-03
<i>lmo1262</i>		transcriptional regulator	11.09	6.85E-06
<i>lmo0371</i>		GntR family transcriptional regulator	10.93	7.36E-07
<i>lmo0229</i>		CtsR family transcriptional regulator	9.20	5.96E-04
<i>lmo0651</i>		transcriptional regulator	8.16	5.12E-04
<i>lmo0031</i>		LacI family transcription regulator	7.36	1.56E-05
<i>lmo2493</i>		ArsR family transcriptional regulator	6.61	7.09E-05
ABC transporter				
<i>lmo2115</i>	<i>anrB</i>	ABC transporter permease	157.10	2.10E-10
<i>lmo2114</i>	<i>anrA</i>	ABC transporter ATP-binding protein	131.39	4.47E-07
<i>lmo1746</i>	<i>virB</i>	ABC transporter permease	35.71	2.03E-06
<i>lmo2768</i>		hypothetical protein	21.54	1.40E-06
<i>lmo2769</i>		ABC transporter ATP-binding protein	13.72	4.47E-07
<i>lmo1740</i>		amino acid ABC transporter permease	13.62	7.17E-05
<i>lmo2125</i>		sugar ABC transporter substrate-binding protein	7.55	3.42E-05
Phosphotransferase system (PTS)				
<i>lmo0916</i>		PTS sugar transporter subunit IIA	16.50	4.85E-05
<i>lmo0024</i>		PTS mannose transporter subunit IID	7.49	1.97E-05
<i>lmo2649</i>	<i>ulaA</i>	PTS system ascorbate transporter subunit IIC	6.61	9.65E-06
<i>lmo1971</i>	<i>ulaA</i>	PTS ascorbate transporter subunit IIC	6.09	2.50E-02
<i>lmo0021</i>		PTS fructose transporter subunit IIA	5.52	7.17E-05
<i>lmo0301</i>		PTS beta-glucoside transporter subunit IIA	5.01	1.87E-03
DNA repair				
<i>lmo1320</i>	<i>polC</i>	DNA polymerase III PolC	6.47	3.73E-02
<i>lmo1275</i>	<i>topA</i>	DNA topoisomerase I	6.04	3.37E-03
<i>lmo2523</i>		single-strand DNA-binding protein	5.26	7.09E-05
Secondary metabolites synthesis				
<i>lmo1809</i>	<i>plsX</i>	glycerol-3-phosphate acyltransferase PlsX	390.97	3.78E-10
<i>lmo2212</i>	<i>hemE</i>	uroporphyrinogen decarboxylase	25.56	2.50E-04
<i>lmo1557</i>	<i>hemA</i>	glutamyl-tRNA reductase	24.91	7.28E-07
<i>lmo2211</i>	<i>hemH</i>	ferrochelatase	8.87	4.58E-02

<i>lmo0884</i>		protoporphyrinogen oxidase	7.57	2.03E-04
<i>lmo1556</i>	<i>hemC</i>	porphobilinogen deaminase	6.45	4.51E-03
<i>lmo1932</i>		heptaprenyl diphosphate synthase subunit I	6.01	7.39E-03
<i>lmo2113</i>		heme peroxidase	5.37	1.39E-03
Oxidative phosphorylation				
<i>lmo2535</i>	<i>atpB</i>	ATP synthase F0F1 subunit A	69.11	7.19E-08
<i>lmo2531</i>	<i>atpA</i>	ATP synthase F0F1 subunit alpha	44.08	4.13E-06
<i>lmo2532</i>	<i>atpH</i>	ATP synthase F0F1 subunit delta	20.30	4.03E-05
<i>lmo2530</i>	<i>atpG</i>	ATP synthase F0F1 subunit gamma	15.92	1.71E-05
<i>lmo2529</i>	<i>atpD</i>	ATP synthase F0F1 subunit beta	9.63	5.96E-05
Others				
<i>lmo1229</i>		hypothetical protein	415.25	6.85E-06
<i>lmo2485</i>		hypothetical protein	396.50	2.10E-10
<i>lmo1249</i>		hypothetical protein	191.15	1.14E-04
<i>lmo1967</i>	<i>tela</i>	toxic ion resistance protein	114.52	2.22E-08
<i>lmo1841</i>		hypothetical protein	93.01	1.02E-02
<i>lmo1966</i>		hypothetical protein	90.88	9.01E-07
<i>lmo0290</i>	<i>yycI</i>	hypothetical protein	63.11	4.16E-09
<i>lmo2503</i>		cardiolipin synthase	41.87	4.11E-08
<i>lmo2811</i>	<i>trmE</i>	tRNA modification GTPase TrmE	26.17	2.04E-03
<i>lmo2156</i>		hypothetical protein	25.13	9.20E-07
<i>lmo0289</i>	<i>yycH</i>	hypothetical protein	23.84	4.47E-07
<i>lmo1757</i>		hypothetical protein	20.46	9.40E-07
<i>lmo1496</i>	<i>greA</i>	transcription elongation factor GreA	18.95	2.73E-02
<i>lmo2473</i>		hypothetical protein	18.31	7.73E-07
<i>lmo1289</i>		internalin	14.77	3.08E-05
<i>lmo0261</i>		phospho-beta-glucosidase	14.59	2.27E-07
<i>lmo2041</i>	<i>mraW</i>	S-adenosyl-methyltransferase MraW	12.43	4.44E-05
<i>lmo2077</i>		glycoprotease	12.23	2.22E-02
<i>lmo0167</i>		hypothetical protein	10.81	1.32E-04
<i>lmo2468</i>	<i>clpP</i>	ATP-dependent Clp protease proteolytic subunit	10.53	2.48E-02
<i>lmo1215</i>		N-acetylmuramoyl-L-alanine amidase	10.22	2.97E-04
<i>lmo2101</i>		pyridoxal biosynthesis lyase PdxS	10.18	4.08E-02
<i>lmo0052</i>		hypothetical protein	9.45	2.86E-05
<i>lmo1432</i>		hypothetical protein	9.20	3.72E-02
<i>lmo0737</i>		hypothetical protein	8.70	5.98E-03
<i>lmo0963</i>	<i>htpX</i>	heat shock protein HtpX	8.04	7.28E-06
<i>lmo1272</i>	<i>rbgA</i>	ribosomal biogenesis GTPase	7.67	2.29E-04
<i>lmo1888</i>		hypothetical protein	7.34	1.14E-06
<i>lmo0072</i>		hypothetical protein	7.34	2.19E-04
<i>lmo1842</i>		hypothetical protein	7.00	6.48E-03

<i>lmo1854</i>		hypothetical protein	6.72	7.87E-06
<i>lmo1484</i>	<i>comEA</i>	competence protein ComEA	6.46	7.17E-04
<i>lmo1820</i>		serine/threonine protein kinase	6.33	8.79E-05
<i>lmo0962</i>	<i>lemA</i>	LemA protein	6.23	6.00E-05
<i>lmo1598</i>	<i>tyrS</i>	tyrosyl-tRNA synthetase	5.77	1.08E-02
<i>lmo1800</i>		protein-tyrosine phosphatase	5.77	1.01E-04
<i>lmo0583</i>	<i>azi, div</i>	preprotein translocase subunit SecA	5.69	6.54E-05
<i>lmo0954</i>		hypothetical protein	5.61	1.89E-04
<i>lmo2484</i>		hypothetical protein	5.48	7.28E-06
<i>lmo2406</i>		hypothetical protein	5.48	1.93E-04
<i>lmo02333</i>		hypothetical protein	5.40	1.14E-04

Table S3. *L. monocytogenes* genes related to nisin sensitivity as identified by Tn-seq analysis.

ORF	Gene	Product	Fold	BH
<i>Lmo0215</i>		hypothetical protein	0.15	7.28E-07
<i>lmo0653</i>		hypothetical protein	0.14	3.23E-05
<i>lmo0955</i>		hypothetical protein	0.11	3.51E-05
<i>lmo1464</i>		diacylglycerol kinase	0.06	6.85E-06
<i>lmo2258</i>		hypothetical protein	0.09	2.92E-07
<i>lmo2380</i>		monovalent cation/H ⁺ antiporter subunit C	0.11	2.50E-02
<i>lmo2549</i>	<i>gtcA</i>	wall teichoic acid glycosylation protein GtcA	0.15	1.21E-05

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