

Supplemental information

Lemon exosome-like nanoparticles enhance stress survival of gut bacteria by RNase P-mediated specific tRNA decay

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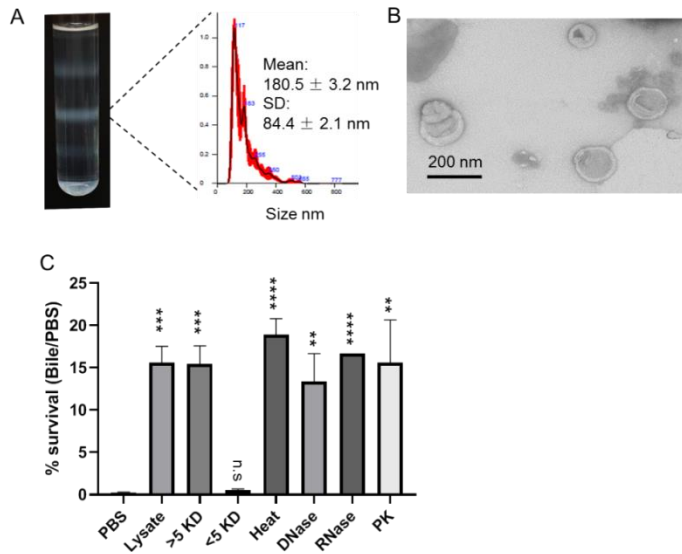


Fig. S1 Characterization of LELNs and identification of active factors that increase the bile resistance of LGG. (A) Size distribution of LELNs. (B) EM image of LELNs. (C) fraction and identification of LELNs effect factor, lysate indicates LELNs lysate by sonication; heat indicates heat lysate for 30 min at 80°C, >5 KD and < 5KD indicate lysate separated using a 5 KD membrane filter, DNase, RNase and PK indicate DNase I, RNase A and Protease K treatments, respectively, for 1 h at 37°C. PBS was used as a control. **Related to Fig. 1.**

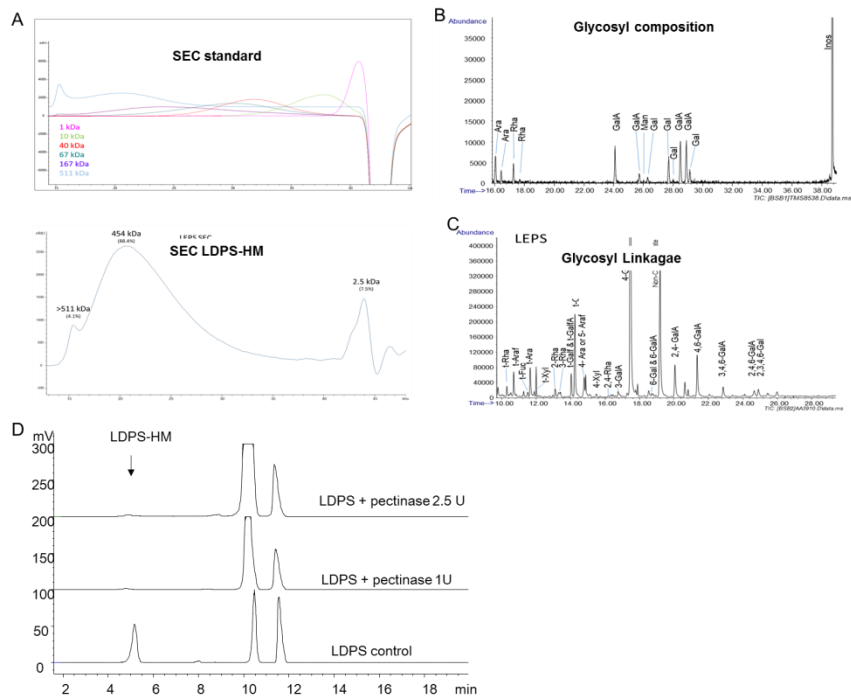


Fig. S2 Characterization of LELNs derived pectin. (A) Molecular weight determination, (B) Glycosyl composition and (C) Glycosyl linkage analysis of LELN derived pectin. (D) Pectinase digest test of LELN

derived pectin, reaction was performed at room temperature for 1h in acetate buffer, pH4.5. **Related to Fig. 1.**

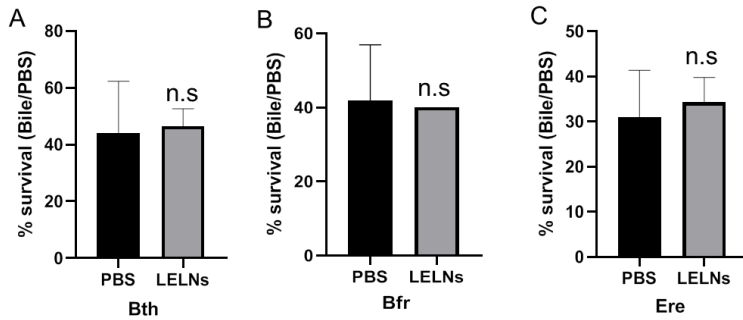


Fig. S3 Bile resistance test of bacteroides and eubacterium strains. Bth represents *Bacteroides thetaiotaomicron* ATCC29418, Bfr represents *Bacteroides fragilis* NCTC9343 and Ere represents *Eubacterium rectale* ATCC33656. **Related to Fig. 1.**

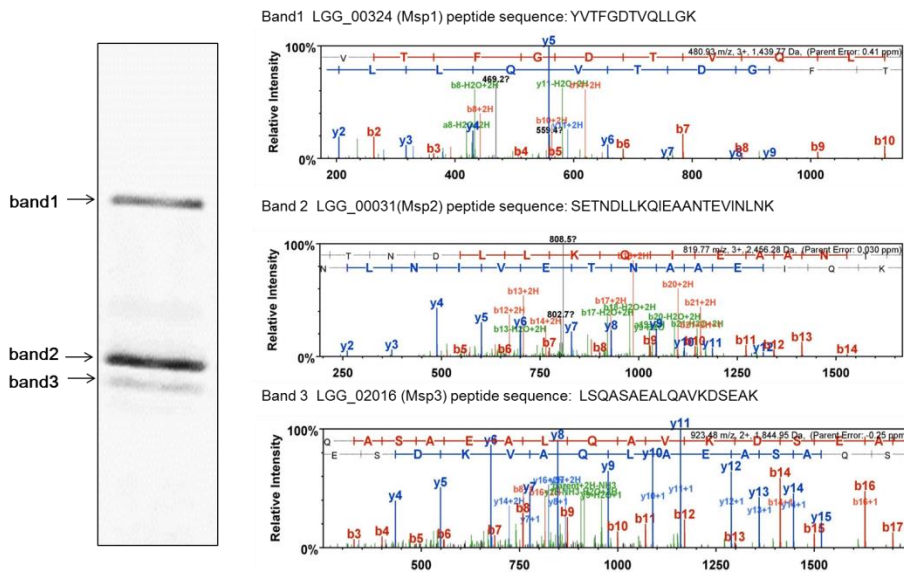


Fig. S4 MALDI-TOF Mass confirmed major proteins in LGG culture supernatant. **Related to Fig. 2.**

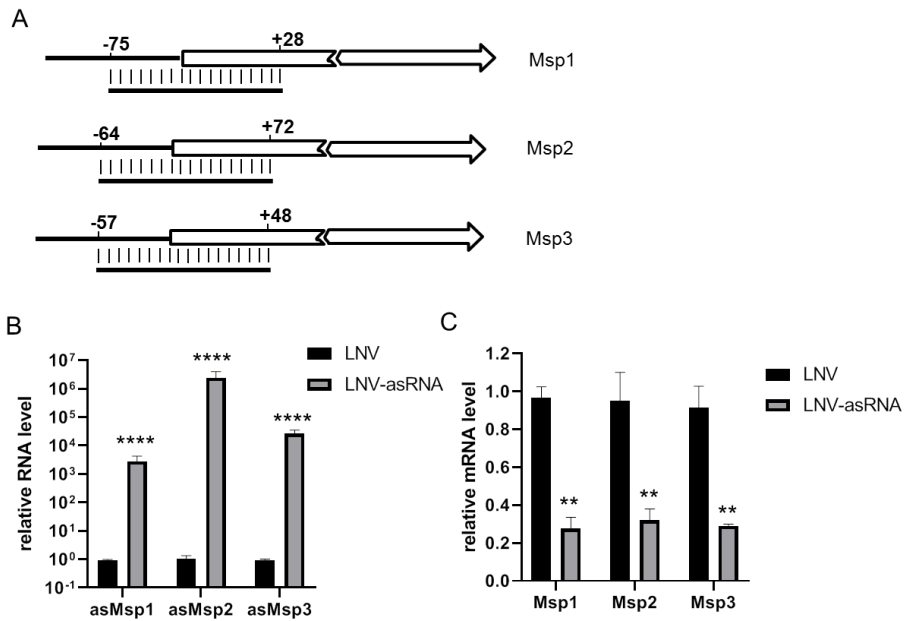


Fig. S5 Knockdown Msp by antisense RNA. (A) Diagram of Msp antisense RNA designs, +1 was set as the first nucleotide of the coding sequence. (B) Antisense RNA transformation efficiency. (C) qRT-PCR analysis of *msp1*, *msp2* and *msp3* mRNA levels in both LNV control and knockdown groups. Bacteria were collected at 4 h after adding antisense RNA. **Related to Fig. 2.**

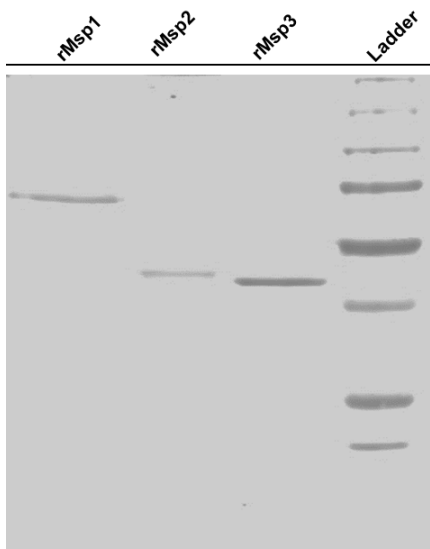


Fig. S6 SDS-PAGE confirm to heterologous expression and purification of rMsp1, rMsp2, and rMsp3. Related to Fig. 2.

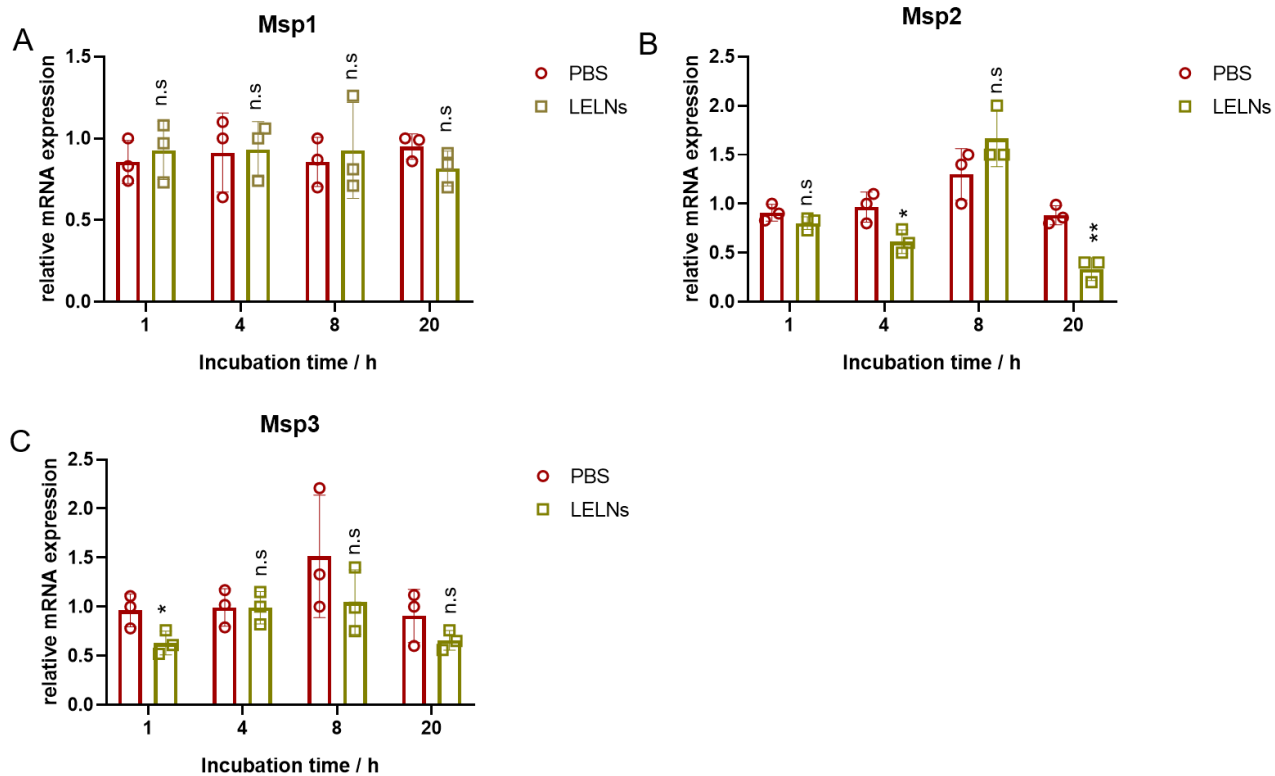


Fig. S7 mRNA level of Msp1 (A), Msp2 (B) and Msp3 (C) with or without LELN treatment. 1×10^{10} /ml LELNs were added into the LGG culture that had reached an OD600=0.6; bacteria were collected at 4 different time points as indicated after adding LELNs. **Related to Fig. 3.**

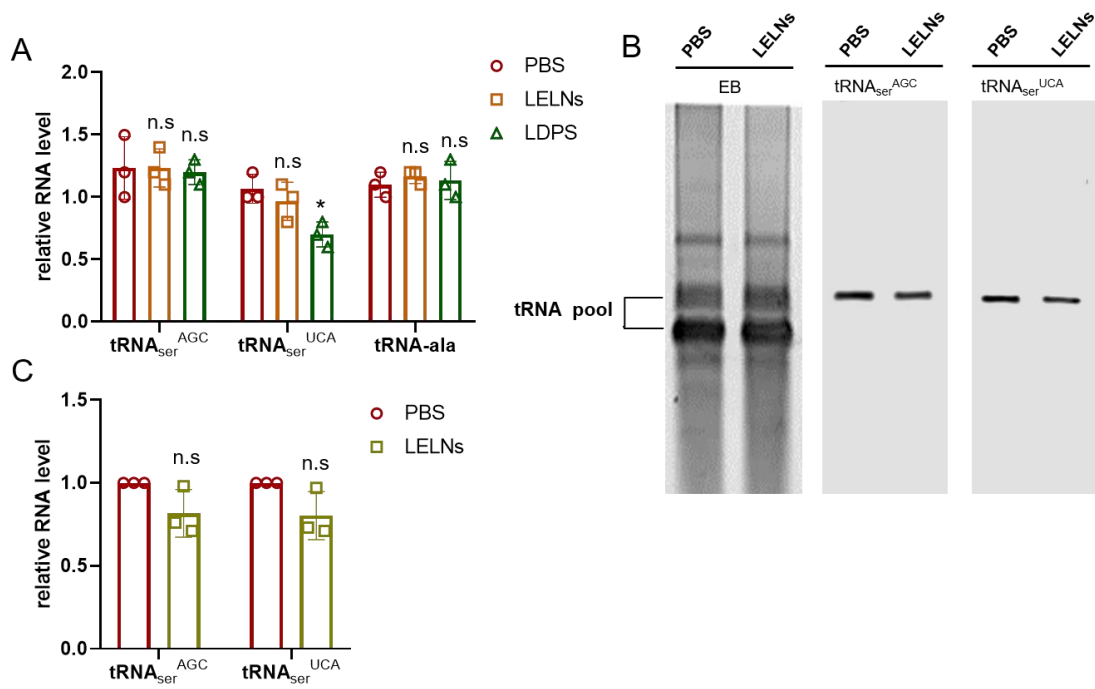


Fig. S8 Alanine and serine tRNA levels in LGG. 1×10^{10} /ml LELNs were added to LGG cultures that had reached an OD₆₀₀=0.6 and cells were harvested 4 h after adding LELNs. Serine tRNA levels were detected by qRT-PCR (A) and Northern blot (B and C). **Related to Fig. 3.**

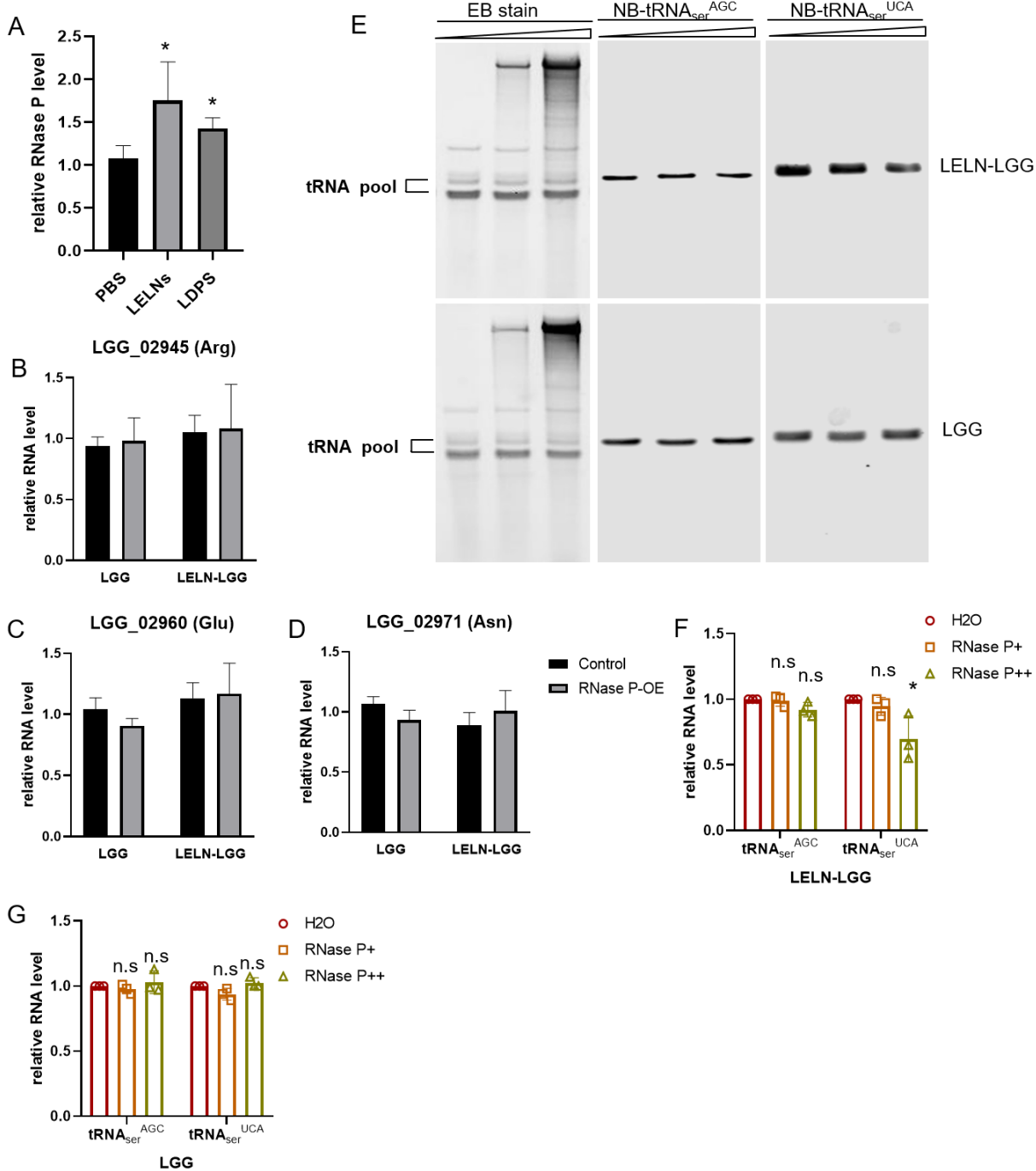


Fig. S9 *In vitro* RNase P decay analysis of tRNA_{ser}^{AGC} and tRNA_{ser}^{UCA}. (A) qRT-PCR analysis of RNase P levels in LGG. 1 x 10¹⁰ /ml LELNs or 10 mg/ml LDPS were added to LGG when it reached an OD₆₀₀=0.6 and bacteria were harvested 4 h after treatment. (B-D) RT-PCR analyses of tRNAs as indicated in RNase overexpression and control strain. (E-G) *In-vitro* RNase P cleavage assay of

tRNA_{ser}^{AGC} and tRNA_{ser}^{UCA} from LGG or LELN-LGG. Two different doses of RNase P, 200 ng (middle lanes or RNase P+) and 2 µg (right lanes or RNase P++) were used in the cleavage assay. H₂O treatment was used as a control (left lanes), EB stain was used as a loading control. Northern blots were quantified using Image J software. **Related to Fig. 4.**

Tables

Table S1 Glycosyl composition of LELNs derived pectin. Related to Fig. 1.

Glycosyl residue	Mass (µg)	Mol % ¹
Arabinose (Ara)	4.3	10.7
Rhamnose (Rha)	1.6	3.7
Galacturonic acid (GalA)	38.5	74.4
Mannose (Man)	0.2	0.4
Galactose (Gal)	5.2	10.9
SUM	49.7	100.1

¹The total Mol % may not add to exactly 100% due to rounding.

Table S2 Glycosyl Linkage of LELNs derived pectin. Related to Fig. 1.

Peak	Percent area
Terminal Rhamnopyranosyl residue (t-Rha)	1.33
Terminal Arabinofuranosyl residue (t-Araf)	3.15
Terminal Fucopyranosyl residue (t-Fuc)	0.56
Terminal Arabinopyranosyl residue (t-Ara)	4.35
Terminal Xylopyranosyl residue (t-Xyl)	0.78
2 linked Rhamnopyranosyl residue (2-Rha)	1.27
3 linked Rhamnopyranosyl residue (3-Rha)	0.89
Terminal Galactofuranosyl residue (t-Galf)	0.35
Terminal Galactofuranosyl uronic acid residue (t-GalfA)	2.93
Terminal Galactopyranosyl residue (t-Gal)	12.10
Terminal Galactopyranosyl uronic acid residue (t-GalA)	0.77

4 linked Arabinopyranosyl or 5 linked Arabinofuranosyl residue (4-Ara or 5-Araf)	0.57
4 linked Xylopyranosyl residue (4-Xyl)	0.50
4 linked Rhamnopyranosyl residue (4-Rha)	0.21
3 linked Galactopyranosyl uronic acid residue (3-GalA)	0.94
4 linked Galactopyranosyl uronic acid residue (4-GalA)	57.37
6 linked Galactopyranosyl residue (6-Gal)	0.11
2,4 linked Galactopyranosyl uronic acid residue (2,4-GalA)	8.35
3,4 linked Galactopyranosyl uronic acid residue (3,4-GalA)	1.94
2,3,4,6 linked Galactopyranosyl residue (2,3,4,6-Gal)	1.53
Sum	100

Table S3 Top 8 upregulated genes (red) and top 11 downregulated genes (green) due to LELNs treatment detected by MALTI-TOF MASS. Related to Fig. 2.

Locus	symbol	annotation	pathway	serine%	alanine%
LGG_01056	Map	Methionine aminopeptidase	Peptidases	3.05	8.63
LGG_00769	Alars	Conserved protein	unknown	2.33	4.65
LGG_01459	pyrB	Aspartate carbamoyltransferase	Nucleotide metabolism	6.07	8.95
LGG_01603	DnaJ	Chaperone protein	Chaperones and folding catalysts	3.83	9.33
LGG_02039	RlmC	dTDP-4-dehydrorhamnose 3,5-epimerase	Metabolism of terpenoids and polyketides	3.68	5.79
LGG_01015	PcrA	ATP-dependent DNA helicase	Replication and repair	4	10.8
LGG_02357		CsbD-like superfamily protein	unknown, stress responding protein	5.8	11.59
LGG_00856	BsaA	Glutathione peroxidase	Glutathione metabolism	4.46	8.92
LGG_00763	YtqI	Phosphoesterase, DHH family protein	Energy metabolism	5.35	11.01
LGG_01171	PrfA	Peptide chain release factor 1	Translation factors	5.57	6.13
LGG_02466	Adk	Adenylate kinase	Nucleotide metabolism	1.83	10.55
LGG_01311	YkqC	Metallo-beta-lactamase superfamily protein	RNA degradation	5.36	7.32
LGG_02120	FabH	3-oxoacyl-[acyl-carrier-protein] synthase III	Fatty acid biosynthesis	6.58	12.54
LGG_01945	OppA	ABC transporter	transporter	7.91	7.55
LGG_00208		Conserved protein	unknown	4.77	10.34
LGG_02255		Conserved hypothetical membrane spanning protein	unknown	7.56	9.88
LGG_00324	Msp1	Cell wall-associated glycoside hydrolase	Peptidoglycan biosynthesis and degradation	10.44	18.05
LGG_00031	Msp2	Surface antigen	Peptidoglycan biosynthesis and degradation	15.63	15.14
LGG_02016	Msp3	Surface antigen (NLP/P60)	Peptidoglycan biosynthesis and degradation	12.78	24.1

Table S4 Upregulated and downregulated genes due to LELNs treatment detected by RNA-seq. Related to Fig. 4.

Annotation	Gene Symbol	FPKM_PBS	FPKM_LELN	Fold (LELN/PBS)
Malolactic regulator	LGG_RS15290	0	31.41	#DIV/0!
Malolactic regulator	LGG_RS14165	0	63.27	#DIV/0!
ATP-dependent nuclease subunit B	LGG_RS14380	0	26.89	#DIV/0!
Malolactic regulator	LGG_RS14965	0	49.88	#DIV/0!
Malolactic regulator	LGG_RS15285	0	10.04	#DIV/0!
hypothetical protein	LGG_RS14600	0	21.61	#DIV/0!
DNA methyltransferase	LGG_RS14195	0	20.1	#DIV/0!
Malolactic regulator	LGG_RS14955	0	14.41	#DIV/0!
acetyltransferase	LGG_RS15040	0	18.48	#DIV/0!
hypothetical protein	LGG_RS15390	0	14.52	#DIV/0!
hypothetical protein	LGG_RS15240	3.83	38.12	9.96
alpha-galactosidase	LGG_RS15415	1.95	14.53	7.47
hypothetical protein	LGG_RS15420	2.67	19.92	7.47
hypothetical protein	LGG_RS15450	2.94	14.65	4.98
Malolactic regulator	LGG_RS15350	9.31	34.75	3.73
ABC transporter ATP-binding protein	LGG_RS00165	6.72	24.23	3.61
ATP-dependent nuclease subunit B	LGG_RS14765	19.26	65.92	3.42
aspartate carbamoyltransferase catalytic subunit	LGG_RS06995	112.28	346.22	3.08
carbamoyl-phosphate synthase large subunit	LGG_RS06980	117.63	355.09	3.02
carbamoyl phosphate synthase small subunit	LGG_RS06985	115.27	346.28	3
dihydroorotase	LGG_RS06990	102.69	303.67	2.96
ABC transporter permease	LGG_RS00160	6.44	18.79	2.92
ATP-dependent nuclease subunit B	LGG_RS14375	7.28	21.15	2.9
orotidine-5'-phosphate decarboxylase	LGG_RS06970	137.87	375.33	2.72
dihydroorotate dehydrogenase	LGG_RS06975	139.1	369.63	2.66
DNA methyltransferase	LGG_RS14915	8.66	21.56	2.49
msp3	LGG_RS09710	85.18	207.76	2.44
RNase P	LGG_RS14885	110671	262808	2.37
bifunctional pyr operon transcriptional regulator/uracil phosphoribosyltransferase PyrR	LGG_RS07005	110.89	261.56	2.36
uracil transporter	LGG_RS07000	88.95	207.86	2.34
hypothetical protein	LGG_RS11175	45.05	104.37	2.32
hypothetical protein	LGG_RS15075	10.2	23.58	2.31
hypothetical protein	LGG_RS14855	16.01	36.53	2.28
orotate phosphoribosyltransferase	LGG_RS06965	158.26	356.21	2.25
50S ribosomal protein L33	LGG_RS09160	3493.53	7741.48	2.22
prevent-host-death protein	LGG_RS14285	243.78	531.04	2.18
hypothetical protein	LGG_RS15205	25.37	54.14	2.13
putative holin-like toxin	LGG_RS15165	23.18	49.05	2.12
transposase	LGG_RS09450	15.56	32.78	2.11

hypothetical protein	LGG_RS14645	39.87	81.21	2.04
XkdX family protein	LGG_RS14905	58.32	29.04	0.5
beta-hydroxyacyl-ACP dehydratase	LGG_RS10215	365.45	181.42	0.5
acetyltransferase	LGG_RS14980	290.2	141.35	0.49
iron-sulfur cluster biosynthesis family protein	LGG_RS13425	14458.3	7033.43	0.49
hypothetical protein	LGG_RS00135	58.67	28.4	0.48
acetyltransferase	LGG_RS14215	355.5	170.2	0.48
2-nitropropane dioxygenase	LGG_RS10195	207.24	97.79	0.47
acetyltransferase	LGG_RS14210	340.66	157.06	0.46
beta-ketoacyl-[acyl-carrier-protein] synthase II	LGG_RS10180	106.69	48.03	0.45
acetyl-CoA carboxylase subunit beta	LGG_RS10160	205.88	92.42	0.45
3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	LGG_RS10170	173.78	77.48	0.45
histidinol-phosphate transaminase	LGG_RS06930	112.95	50.06	0.44
hypothetical protein	LGG_RS12910	58.03	25.13	0.43
hypothetical protein	LGG_RS14290	903.95	388.87	0.43
ketoacyl-ACP synthase III	LGG_RS10205	195.47	83.8	0.43
TetR/AcrR family transcriptional regulator	LGG_RS02330	18.24	7.8	0.43
acetyl-CoA carboxylase biotin carboxylase subunit	LGG_RS10165	150.11	63.98	0.43
hypothetical protein	LGG_RS14360	24.28	10.08	0.41
hypothetical protein	LGG_RS14790	19.56	8.12	0.41
hypothetical protein	LGG_RS14415	24.01	9.96	0.41
CAAX protease family protein	LGG_RS14140	30.05	12.47	0.41
Malolactic regulator	LGG_RS15140	32.62	13.53	0.41
acetyl-CoA carboxylase carboxyl transferase subunit alpha	LGG_RS10155	117.19	48.38	0.41
3-oxoacyl-ACP reductase FabG	LGG_RS10185	139.61	54.74	0.39
Malolactic regulator	LGG_RS14550	330.37	126.53	0.38
ACP S-malonyltransferase	LGG_RS10190	154.11	55.43	0.36
hypothetical protein	LGG_RS14680	25.37	9.02	0.36
hypothetical protein	LGG_RS14875	21.67	7.71	0.36
hypothetical protein	LGG_RS14800	11.57	4.12	0.36
MarR family transcriptional regulator	LGG_RS10210	298.23	101.88	0.34
transposase	LGG_RS02175	22.33	7.58	0.34
metal ABC transporter substrate-binding protein	LGG_RS11595	705.38	234.5	0.33
hypothetical protein	LGG_RS15370	60.23	18.74	0.31
ATP-dependent nuclease subunit B	LGG_RS15105	67.2	20.19	0.3
hypothetical protein	LGG_RS09520	55.41	16.23	0.29
IMP cyclohydrolase	LGG_RS15235	74.23	18.48	0.25
hypothetical protein	LGG_RS02085	14.68	2.15	0.15
hypothetical protein	LGG_RS14410	14.5	0	0
Malolactic regulator	LGG_RS14675	23.33	0	0
ATP-dependent nuclease subunit B	LGG_RS15365	37.01	0	0
ATP-dependent nuclease subunit B	LGG_RS14425	33.23	0	0

Table S5 Primers used in this research. Related to STAR METHODS

Names	Sequences	Usages
msp1-RTF	AACATGGTATCGGCTTGCCA	qRT-PCR
msp1-RTR	GCAGCGGCAGAATCATTAGC	qRT-PCR
msp2-RTF	AGACGCTTTGGATGCGGTAA	qRT-PCR
msp2-RTR	CTTGCTTATCGCTTGGGTC	qRT-PCR
msp3-RTF	ATGGCTTAAAGGCGGATGCT	qRT-PCR
msp3-RTR	GCTAGCGGTGGAAGTAGCAA	qRT-PCR
Msp1-EF	AAAATCTATACTTCCAAGGAATGGTAGATTCTAAGAAAGTATTG	PCR amplify for heterologous expression
Msp1-ER	CTTCCTTTCGGGCTTGTATTATAGTGACGGGCGAACCGC	PCR amplify for heterologous expression
Msp2-EF	ATCATCATCACAGCAGCGGCAAATTAATAAAGCAATGATGACA	PCR amplify for heterologous expression
Msp2-ER	CTTCCTTTCGGGCTTGTATTACCGGTGGATGTAAACGTAG	PCR amplify for heterologous expression
Msp3-EF	ATCATCATCACAGCAGCGGCGAGATTAAAGCAATTGGTTACGGG	PCR amplify for heterologous expression
Msp3-ER	CTTCCTTTCGGGCTTGTATTAGAGACGACGGCCGAAGC	PCR amplify for heterologous expression
PET28b-MBP-TEV-F	TAACAAAGCCCGAAAGGAAGC	Linearize pET28b-MBP-TEV vector
PET28b-MBP-TEV-R	TCCTTGGAAGTATAGATTTTCACC	Linearize pET28b-MBP-TEV vector
PET28b-R	GCCGCTGCTGTGATGATGATG	Linearize pET28b vector
anti-msp1-F	TAATACGACTCACTATAGGGCTGACAATACTTTCTTAGAATCTACC	in vitro transcription of antisense RNA
anti-msp1-R	GAAAAGGTAAATGCAAATTAAGTG	in vitro transcription of antisense RNA
anti-msp2-F	TAATACGACTCACTATAGGGTGTAAAGGCGCTAACAGAACCC	in vitro transcription of antisense RNA
anti-msp2-R	ATGGTGACGTAACATTGACAC	in vitro transcription of antisense RNA
anti-msp3-F	TAATACGACTCACTATAGGGTAATGTTGCAACCGTGATGGC	in vitro transcription of antisense RNA
anti-msp3-R	ACAGTTCAGTGTTAGTGTGAACC	in vitro transcription of antisense RNA
tRNA-ala-F	TCAGTTGGGAGAGCGCCTG	qRT-PCR alanine tRNA
tRNA-ala-R	ACTTGAACCGTCGACCTCT	qRT-PCR alanine tRNA
SerAGC-RT-F	ACTCAAGTGGCTGAAGAGGC	qRT-PCR serine tRNA
SerAGC-RT-R	GAGGGATTTGAACCCCTCGCA	qRT-PCR serine tRNA
SerUCA-RT-F	CCAAGTCTGGCTGAAGGGAA	qRT-PCR serine tRNA
SerUCA-RT-R	GGATTCGAACCCCTCGCGT	qRT-PCR serine tRNA
SerUCC-RT-F	GTTGTCCGAGAGGCTGAAGG	qRT-PCR serine tRNA
SerUCC-RT-R	TTCGAACCCCTTGATACAGGCA	qRT-PCR serine tRNA
SerUCG-RT-F	GGTATTAGCCGGTTCGACGG	qRT-PCR serine tRNA
SerUCG-RT-R	GGCAGAGTGGTAATGCAACG	qRT-PCR serine tRNA
anti-SerAGC-F	TAATACGACTCACTATAGGGAGAAGGAGGGATTGTAAC	in vitro transcription of antisense RNA
anti-SerAGC-R	GGAGAAGTACTCAAGTGGCTG	in vitro transcription of antisense RNA
anti-SerUCA-F	TAATACGACTCACTATAGGGAGAATGAGGGATTGTAAC	in vitro transcription of antisense RNA
anti-SerUCA-R	GGAGAATTACCCAAGTCTGGC	in vitro transcription of antisense RNA
anti-SerUCC-F	TAATACGACTCACTATAGGGAGAGTAAGGGATTGTAAC	in vitro transcription of antisense RNA

anti-SerUCC-R	AGAGTTGTCCGAGAGGCTG	in vitro transcription of antisense RNA
anti-SerUCG-F	TAATACGACTCACTATAGGGAGAGTACAGGATTTGAACCTGC	in vitro transcription of antisense RNA
anti-SerUCG-R	AGAGTTGGCAGAGTGTAATGC	in vitro transcription of antisense RNA
SerAGC-p	Bio-CAGTTTCCCGTCTACACCCTTAGCAGGGG	Probes for northern blot
SerUCA-p	Bio-GATTGACATCCACCTGACGGTTTTCAAGACC	Probes for northern blot
SerUCC-p	Bio-GGCAAACCCGTATACATGGTTTTCCAACCAT	Probes for northern blot
SerUCG-p	Bio-GTATTAGCCGGTTCGACGGATTTGAGTCC	Probes for northern blot
LGG-rpoD-F	ACAAGGCGATCAAGAAGCCA	qRT-PCR for LGG Quantification
LGG-rpoD-R	CAGAAACTGCATGCCACGAC	qRT-PCR for LGG Quantification
Lactobacillaceae-F	AGCAGTAGGGAATCTTCCA	qRT-PCR for <i>in vitro</i> culture microbiota
Lactobacillaceae-R	CACCGCTACACATGGAG	qRT-PCR for <i>in vitro</i> culture microbiota
U-926F	AAACTCAAAGAATTGACGG	qRT-PCR for <i>in vitro</i> culture microbiota
U-1062R	CTCACRRACGAGCTGAC	qRT-PCR for <i>in vitro</i> culture microbiota