

Molecular Omics

Supplementary material

Proteomic analysis of *Listeria monocytogenes* exposed to free and nanostructured antimicrobial lipopeptides

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Supplementary Table S1

Table S1. PLS-DA values used to identify differentially expressed proteins related to the exposure of *L. monocytogenes* to empty nanoliposome, free and nano-encapsulated peptide P34, with the VIP threshold > 1.0 in the first component of PLS-DA.

Protein name	Gene name	VIP Score		
		Comp. 1	Comp. 2	Comp. 3
DNA protection during starvation protein	<i>dps</i>	5.41	5.18	5.14
Aldehyde-alcohol dehydrogenase	<i>lmo1634</i>	5.16	4.84	4.81
Lmo1257 protein	<i>lmo1257</i>	4.83	4.57	4.54
Manganese-binding lipoprotein MntA	<i>mntA</i>	4.26	4.29	4.26
Pyruvate formate-lyase	<i>pflB</i>	3.99	3.84	3.81
FruA protein	<i>fruA</i>	3.95	3.80	3.78
Manganese transport system ATP-binding protein MntB	<i>mntB</i>	3.58	3.74	3.71
Lmo0135 protein	<i>lmo0135</i>	3.49	3.27	3.27
Dihydrolipoyl dehydrogenase	<i>lmo1371</i>	3.49	3.29	3.27
Lmo1959 protein	<i>lmo1959</i>	3.29	3.14	3.13
Peptide chain release factor 3	<i>prfC</i>	3.16	2.96	2.94
Translation initiation factor IF-1	<i>infA</i>	2.92	2.80	2.78
Catalase	<i>kat</i>	2.89	2.71	2.70
Uncharacterized protein Lmo0216	<i>lmo0216</i>	2.86	2.68	2.67
Heme-degrading monooxygenase	<i>isdG</i>	2.84	2.67	2.65
Lmo0685 protein	<i>lmo0685</i>	2.77	2.60	2.58
Cysteine synthase	<i>cysK</i>	2.77	2.62	2.60
UDP-N-acetylenolpyruvoylglucosamine reductase	<i>murB</i>	2.77	2.66	2.64
Lmo0663 protein	<i>lmo0663</i>	2.65	2.50	2.49
Lmo0592 protein	<i>lmo0592</i>	2.64	2.63	2.63
UPF0145 protein lmo0208	<i>lmo0208</i>	2.60	2.46	2.44
Pseudouridine synthase	<i>lmo1949</i>	2.58	2.42	2.40
Lmo1719 protein	<i>lmo1719</i>	2.53	2.37	2.36
Bifunctional protein Fold	<i>fold</i>	2.40	2.27	2.27
Lmo2373 protein	<i>lmo2373</i>	2.39	2.27	2.26
ATP-dependent Clp protease proteolytic subunit	<i>clp</i>	2.37	2.23	2.24
DNA-directed RNA polymerase subunit omega	<i>rpoZ</i>	2.34	2.23	2.21

Lmo0152 protein	<i>lmo0152</i>	2.33	2.20	2.19
Lmo0066 protein	<i>lmo0066</i>	2.32	2.18	2.17
Pyridoxal 5-phosphate synthase subunit PdxT	<i>pdxT</i>	2.30	2.16	2.14
UPF0356 protein lmo1028	<i>lmo1028</i>	2.24	2.10	2.09
Phospholipase C	<i>plcB</i>	2.24	2.16	2.16
50S ribosomal protein L35	<i>rpmI</i>	2.21	2.07	2.07
Lmo0541 protein	<i>lmo0541</i>	2.19	2.16	2.17
Tagatose-6-phosphate kinase	<i>fruB</i>	2.19	2.06	2.05
Lmo2752 protein	<i>lmo2752</i>	2.16	2.03	2.01
Lmo2152 protein	<i>lmo2152</i>	2.15	2.11	2.10
Protein DltD	<i>dltD</i>	2.14	2.03	2.01
Lmo2167 protein	<i>lmo2167</i>	2.10	1.97	1.97
Ribulose-phosphate 3-epimerase	<i>lmo1818</i>	2.10	2.00	1.99
30S ribosomal protein S21	<i>rpsU</i>	2.09	1.96	1.96
Lmo1079 protein	<i>lmo1079</i>	2.09	1.96	1.94
Lmo1946 protein	<i>lmo1946</i>	2.05	1.92	1.91
Deoxyuridine 5-triphosphate nucleotidohydrolase	<i>lmo1691</i>	2.04	2.02	2.00
Lmo1812 protein	<i>lmo1812</i>	2.00	1.87	1.86
50S ribosomal protein L32-1	<i>rpmF1</i>	1.99	1.92	1.93
Lmo0955 protein	<i>lmo0955</i>	1.96	1.98	1.97
1-phosphatidylinositol phosphodiesterase	<i>plcA</i>	1.95	1.98	2.02
Lmo2184 protein	<i>lmo2184</i>	1.95	2.05	2.05
Lmo1710 protein	<i>lmo1710</i>	1.88	2.10	2.10
Glucose-1-phosphate thymidyltransferase	<i>lmo1081</i>	1.87	1.77	1.76
UPF0173 metal-dependent hydrolase lmo1577	<i>lmo1577</i>	1.80	1.70	1.69
Transcription termination factor Rho	<i>rho</i>	1.78	1.67	1.66
dTDP-4-dehydrorhamnose 3,5-epimerase	<i>lmo1082</i>	1.77	1.67	1.66
Lmo1510 protein	<i>lmo1510</i>	1.74	1.74	1.73
Lmo2433 protein	<i>lmo2433</i>	1.73	1.71	1.70
Single-stranded DNA-binding protein 1	<i>ssb1</i>	1.73	1.62	1.61
Lmo0982 protein	<i>lmo0982</i>	1.73	1.68	1.67
Lmo0366 protein	<i>lmo0366</i>	1.73	1.66	1.67
Lmo1017 protein	<i>lmo1017</i>	1.72	1.65	1.66
Lmo2209 protein	<i>lmo2209</i>	1.71	1.61	1.60
tRNA-specific 2-thiouridylase MnmA	<i>mnmA</i>	1.70	1.59	1.59
L-lactate dehydrogenase 1	<i>ldh1</i>	1.68	1.63	1.63
Serine acetyltransferase	<i>cysE</i>	1.67	1.87	1.86
Lmo0335 protein	<i>lmo0335</i>	1.66	1.56	1.55
Lmo2569 protein	<i>lmo2569</i>	1.65	1.60	1.60
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2	<i>murA2</i>	1.64	1.60	1.60
ATP synthase gamma chain	<i>atpG</i>	1.64	1.57	1.56
ATP-dependent DNA helicase	<i>pcrA</i>	1.63	1.54	1.53
50S ribosomal protein L36	<i>rpmJ</i>	1.63	1.58	1.57
UPF0154 protein lmo1306	<i>lmo1306</i>	1.60	2.29	2.28
Translation initiation factor IF-3	<i>infC</i>	1.60	1.50	1.49

Lmo0900 protein	<i>lmo0900</i>	1.60	1.79	1.79
HTH-type transcriptional regulator MntR	<i>mntR</i>	1.58	1.57	1.56
Lmo0774 protein	<i>lmo0774</i>	1.58	1.50	1.49
Lmo2168 protein	<i>lmo2168</i>	1.58	1.56	1.55
Glycerol-3-phosphate dehydrogenase	<i>glpD</i>	1.58	1.49	1.48
Putative peptidoglycan bound protein LPXTG motif	<i>lmo0842</i>	1.57	1.91	1.90
Signal peptidase I	<i>lmo1271</i>	1.57	1.48	1.47
Lmo0579 protein	<i>lmo0579</i>	1.53	1.44	1.44
10 kDa chaperonin	<i>groS</i>	1.53	1.44	1.45
Lmo1529 protein	<i>lmo1529</i>	1.50	1.40	1.44
Ribosome hibernation promoting factor	<i>hpf</i>	1.49	1.41	1.42
Lmo0096 protein	<i>lmo0096</i>	1.49	1.54	1.53
FolC protein	<i>folC</i>	1.48	1.52	1.52
Purine nucleoside phosphorylase DeoD-type	<i>deoD</i>	1.47	1.42	1.42
Putative zinc metalloprotease Lmo1318	<i>lmo1318</i>	1.46	1.36	1.35
30S ribosomal protein S13	<i>rpsM</i>	1.44	1.34	1.33
Lmo2208 protein	<i>lmo2208</i>	1.44	1.48	1.46
Lmo1283 protein	<i>lmo1283</i>	1.43	1.34	1.35
Uncharacterized protein Lmo1967	<i>lmo1967</i>	1.42	1.52	1.51
Lmo2199 protein	<i>lmo2199</i>	1.41	1.32	1.32
Lmo0848 protein	<i>lmo0848</i>	1.40	1.43	1.42
30S ribosomal protein S18	<i>rpsR</i>	1.40	1.31	1.33
50S ribosomal protein L18	<i>rplR</i>	1.40	1.31	1.30
Threonine--tRNA ligase	<i>thrS</i>	1.39	1.30	1.30
Regulatory protein Spx	<i>spxA</i>	1.37	1.91	1.90
Lmo1515 protein	<i>lmo1515</i>	1.37	1.32	1.31
50S ribosomal protein L21	<i>rplU</i>	1.37	1.29	1.28
UPF0637 protein lmo1065	<i>lmo1065</i>	1.36	1.29	1.29
Glycerol kinase	<i>glpK</i>	1.36	1.29	1.31
Lmo0257 protein	<i>lmo0257</i>	1.35	1.30	1.29
Lmo0193 protein	<i>lmo0193</i>	1.35	1.29	1.28
Diaminopimelate epimerase	<i>dapF</i>	1.35	1.26	1.26
Glutathione peroxidase	<i>lmo0983</i>	1.34	1.27	1.27
Aminomethyltransferase	<i>gcvT</i>	1.34	1.44	1.44
Lmo0847 protein	<i>lmo0847</i>	1.33	1.32	1.32
Lmo2232 protein	<i>lmo2232</i>	1.32	1.24	1.26
FADprotein FMN transferase	<i>lmo2636</i>	1.31	1.28	1.28
30S ribosomal protein S12	<i>rpsL</i>	1.30	1.24	1.23
Thiol peroxidase	<i>tpx</i>	1.30	1.24	1.23
Lmo0185 protein	<i>lmo0185</i>	1.29	1.22	1.21
Initiation-control protein YabA	<i>lmo0164</i>	1.29	1.48	1.47
tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG	<i>mnmG</i>	1.29	1.20	1.20
Lmo1887 protein	<i>lmo1887</i>	1.28	1.27	1.27
Formate--tetrahydrofolate ligase	<i>fhs</i>	1.28	1.22	1.22
Aspartate--tRNA ligase	<i>aspS</i>	1.28	1.21	1.20

Glucose-6-phosphate 1-dehydrogenase	<i>zwf</i>	1.27	1.23	1.22
Uncharacterized RNA methyltransferase lmo1751	<i>lmo1751</i>	1.26	1.30	1.29
NADPH dehydrogenase	<i>namA</i>	1.26	1.22	1.21
Lmo0734 protein	<i>lmo0734</i>	1.25	1.17	1.17
50S ribosomal protein L31 type B	<i>rpmE2</i>	1.24	1.16	1.16
Lmo1094 protein	<i>lmo1094</i>	1.23	1.17	1.17
50S ribosomal protein L20	<i>rplT</i>	1.23	1.15	1.14
Lmo0084 protein	<i>lmo0084</i>	1.23	1.16	1.15
Lmo1820 protein	<i>lmo1820</i>	1.22	1.20	1.19
Lmo2696 protein	<i>lmo2696</i>	1.19	1.11	1.12
Metal-dependent carboxypeptidase	<i>lmo1886</i>	1.18	1.15	1.15
ATP synthase subunit delta	<i>atpH</i>	1.17	1.10	1.10
Lmo2193 protein	<i>lmo2193</i>	1.17	1.14	1.13
Lipoate--protein ligase	<i>lmo0931</i>	1.17	1.24	1.23
AlsS protein	<i>alsS</i>	1.17	1.10	1.09
Lmo1431 protein	<i>lmo1431</i>	1.17	1.10	1.09
tRNA pseudouridine synthase A	<i>truA</i>	1.16	1.14	1.14
CydA protein	<i>cydA</i>	1.15	1.09	1.09
Lmo1415 protein	<i>lmo1415</i>	1.15	1.08	1.07
Lmo1727 protein	<i>lmo1727</i>	1.14	1.13	1.12
Lmo2700 protein	<i>lmo2700</i>	1.14	1.11	1.11
Lmo1030 protein	<i>lmo1030</i>	1.14	1.08	1.08
Alanine dehydrogenase	<i>lmo1579</i>	1.13	1.07	1.06
Lmo1620 protein	<i>lmo1620</i>	1.13	1.07	1.06
GTP cyclohydrolase 1	<i>folE</i>	1.13	1.18	1.17
CydD protein	<i>cydD</i>	1.13	1.08	1.08
Probable transcriptional regulatory protein lmo1535	<i>lmo1535</i>	1.12	1.09	1.08
Probable tautomerase lmo2564	<i>lmo2564</i>	1.12	1.05	1.04
Lmo2537 protein	<i>lmo2537</i>	1.11	1.07	1.06
GTP-sensing transcriptional pleiotropic repressor CodY	<i>codY</i>	1.10	1.03	1.03
Pseudouridine synthase	<i>lmo1843</i>	1.10	1.05	1.04
Lmo2370 protein	<i>lmo2370</i>	1.10	1.05	1.04
30S ribosomal protein S14 type Z	<i>rpsZ</i>	1.10	1.03	1.02
tRNA-dihydrouridine synthase	<i>lmo0227</i>	1.09	1.13	1.12
Bifunctional protein GlmU	<i>glmU</i>	1.09	1.03	1.03
L-lactate dehydrogenase 2	<i>ldh2</i>	1.09	1.03	1.03
DNA polymerase III subunit gamma/tau	<i>dnaX</i>	1.09	1.08	1.07
Lmo1005 protein	<i>lmo1005</i>	1.08	1.05	1.04
Citrate synthase	<i>citZ</i>	1.08	1.01	1.00
30S ribosomal protein S2	<i>rpsB</i>	1.07	1.03	1.03
Lmo1019 protein	<i>lmo1019</i>	1.07	1.02	1.01
Recombination protein RecR	<i>recR</i>	1.07	1.01	1.01

Supplementary Figure S1

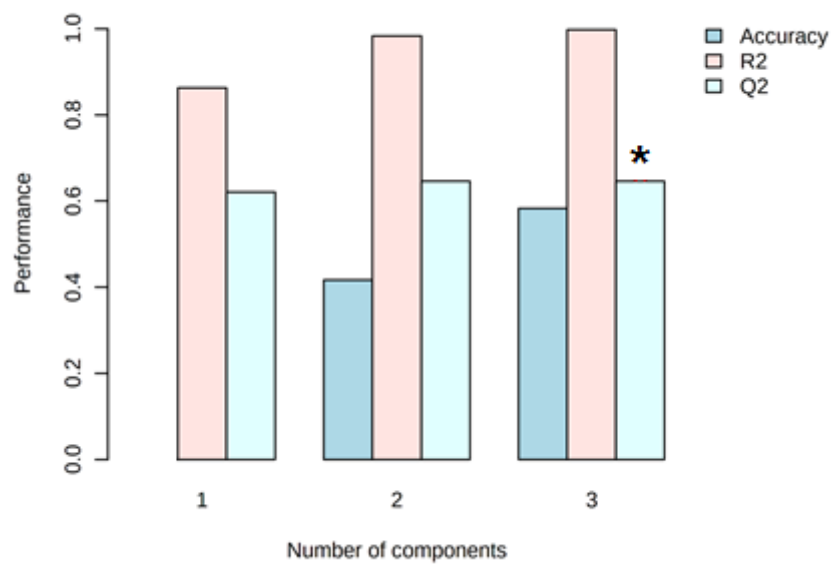


Figure S1. Relation between components and performance of accuracy, R^2 and Q^2 . The red * indicate the highest value for the Q^2 parameter among the components.

Supplementary Table S2

Table S2. List of proteins with VIP ≥ 2 differentially regulated in the treatments with peptide P34. These proteins were used as base for the proteomics study of this work.

Protein name	VIP score	Gene name	Protein regulation
Lmo0135 protein	3.49	<i>lmo0135</i>	Down
Catalase	2.89	<i>kat</i>	Down
Aldehyde-alcohol dehydrogenase	5.16	<i>lmo1634</i>	Down
Pyruvate formate-lyase	3.99	<i>pflB</i>	Down
Manganese-binding lipoprotein MntA	4.25	<i>mntA</i>	Down
Manganese transport system ATP-binding protein MntB	3.58	<i>mntB</i>	Down
Translation initiation factor IF-1	2.91	<i>infA</i>	Down
Peptide chain release factor 3	3.15	<i>prfC</i>	Down
DNA protection during starvation protein	5.41	<i>dps</i>	Down
Lmo1257 protein	4.82	<i>lmo1257</i>	Down
Lmo0592 protein	2.64	<i>lmo0592</i>	Down
Lmo0663 protein	2.65	<i>lmo0663</i>	Down
50S ribosomal protein L35	2.21	<i>rpmI</i>	Down
Lmo2152 protein	2.15	<i>lmo2152</i>	Down

Pyridoxal 5'-phosphate synthase subunit PdxT	2.30	<i>pdxT</i>	Down
Lmo1028 protein	2.24	<i>lmo1028</i>	Down
Lmo0208 protein	2.59	<i>lmo0208</i>	Down
DNA-directed RNA polymerase subunit omega	2.34	<i>rpoZ</i>	Down
Lmo2373 protein	2.39	<i>lmo2373</i>	Down
Lmo1719 protein	2.52	<i>lmo1719</i>	Down
Lmo0152 protein	2.33	<i>lmo0152</i>	Down
Dihydrolipoyl dehydrogenase	3.48	<i>lmo1371</i>	Up
Lmo0685 protein	2.77	<i>lmo0685</i>	Up
FruA protein	3.94	<i>fruA</i>	Up
Uncharacterized protein Lmo0216	2.85	<i>lmo0216</i>	Up
Heme-degrading monooxygenase	2.83	<i>isdG</i>	Up
Lmo1959 protein	3.29	<i>lmo1959</i>	Up
UDP-N-acetylenolpyruvoylglucosamine reductase	2.77	<i>murB</i>	Up
Lmo1079 protein	2.09	<i>lmo1079</i>	Up
Ribulose-phosphate 3-epimerase	2.10	<i>lmo1818</i>	Up
Lmo2752 protein	2.16	<i>lmo2752</i>	Up
Protein DltD	2.14	<i>dltD</i>	Up
30S ribosomal protein S21	2.09	<i>rpsU</i>	Up

Deoxyuridine 5'-triphosphate nucleotidohydrolase	2.04	<i>lmo1691</i>	Up
Tagatose-6-phosphate kinase	2.18	<i>fruB</i>	Up
Lmo1946 protein	2.05	<i>lmo1946</i>	Up
Lmo0066 protein	2.32	<i>lmo0066</i>	Up

Supplementary Table S3

Table S3. Exclusively regulated protein by nanoencapsulated peptide P34. The list proteins with VIP score ≥ 1 .

Protein name	VIP score	Gene name	Protein regulation
Lmo0366 protein	1.73	<i>lmo0366</i>	Up
30S ribosomal protein S18	1.40	<i>rpsR</i>	Up
Lmo2184 protein	1.95	<i>lmo2184</i>	Up
Lmo0579 protein	1.53	<i>lmo0579</i>	Up
Thiol peroxidase	1.30	<i>tpx</i>	Up
Lmo0541 protein	2.19	<i>lmo0541</i>	Up
Lmo1094 protein	1.23	<i>lmo1094</i>	Up
dTDP-4-dehydrorhamnose 3,5-epimerase	1.77	<i>lmo1082</i>	Up
Lmo1620 protein	1.13	<i>lmo1620</i>	Up
Lmo1510 protein	1.74	<i>lmo1510</i>	Up
Lmo2168 protein	1.58	<i>lmo2168</i>	Up
Lmo0185 protein	1.29	<i>lmo0185</i>	Up
Lmo2696 protein	1.19	<i>lmo2696</i>	Up
GTP cyclohydrolase 1	1.17	<i>folE</i>	Up
Pseudouridine synthase	1.10	<i>lmo1843</i>	Up

Lmo2167 protein	2.10	<i>lmo2167</i>	Up
Lmo1005 protein	1.08	<i>lmo1005</i>	Up
Phospholipase C	2.24	<i>plcB</i>	Down
Glycerol-3-phosphate dehydrogenase	1.58	<i>glpD</i>	Down
Lmo0084	1.23	<i>lmo0084</i>	Down
Lmo1710 protein	2.09	<i>lmo1710</i>	Down
50S ribosomal protein L36	1.62	<i>rpmJ</i>	Down
Glycerol kinase	1.36	<i>glpK</i>	Down
Lmo0096 protein	1.49	<i>lmo0096</i>	Down
10 kDa chaperonin	1.53	<i>groS</i>	Down
AlsS protein	1.17	<i>alsS</i>	Down
Ribosome hibernation promoting factor	1.49	<i>hpf</i>	Down
Uncharacterized protein Lmo1967	1.42	<i>lmo1967</i>	Down
UPF0154 membrane protein Lmo1306	2.28	<i>lmo1306</i>	Down

Supplementary Table S4

Table S4. Protein regulation of free and nanoencapsulated P34 proteins group and the exclusively proteins expressed by the treatment with P34 nanoencapsulated according to molecular functions assigned by CELLO2GO algorithm. Molecular functions assigned with smaller percentages in both protein groups are not detailed.

Molecular function	Exclusively N-P34		F-P34 and N-P34	
	Upregulation	Downregulation	Upregulation	Downregulation
Ion binding	21.1%	33.6%	23.9%	20.1%
Oxidoreductase activity	10.8%	16.9%	10.8%	10.2%
Hydrolase activity	8.3%	8.6%	13.1%	3.4%
Kinase activity	2%	8.3%	4.9%	3.6%
Transmembrane transport	-	-	5.1%	10.5%

Supplementary Table S5

Table S5. Protein regulation of free and nanoencapsulated P34 proteins group and the exclusively proteins expressed by the treatment with P34 nanoencapsulated according to biological process assigned by CELLO2GO algorithm. Biological processes assigned with smaller percentages in both protein groups are not detailed.

Biological process	Exclusively N-P34		F-P34 and N-P34	
	Upregulation	Downregulation	Upregulation	Downregulation
Small molecule metabolic process	12%	7.9%	6.5%	5.3%
Cellular nitrogen compound metabolic process	12%	-	4.5%	6.6%
Biosynthetic process	11%	10%	10.6%	9.5%
Response to stress	8.3%	14.9%	5.9%	7.1%
Transport	7.4%	8.3%	12%	14.6%
Homeostatic process	7.4%	-	15.4%	3.3%