

## **Supplementary Information**

### **A Bacteriolysin of *Lactococcus piscium* is potentially involved in mediating contact-dependent antagonism against *Listeria monocytogenes***

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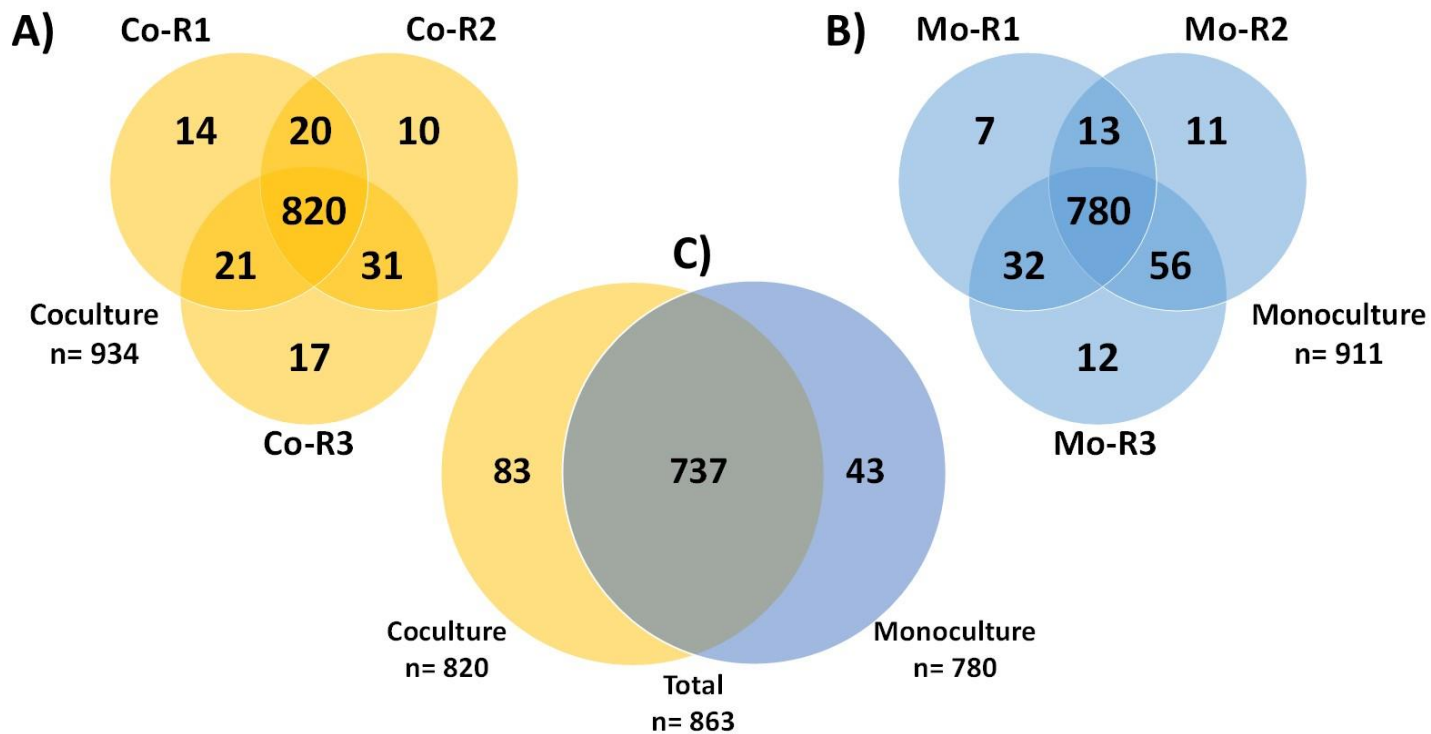
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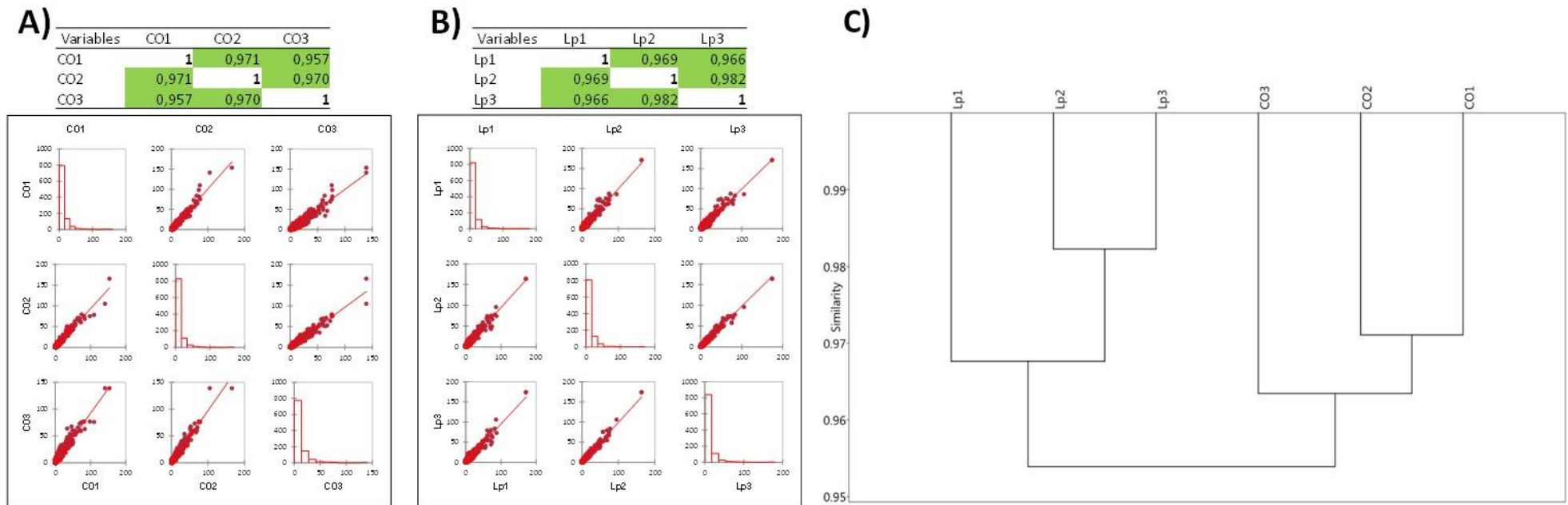
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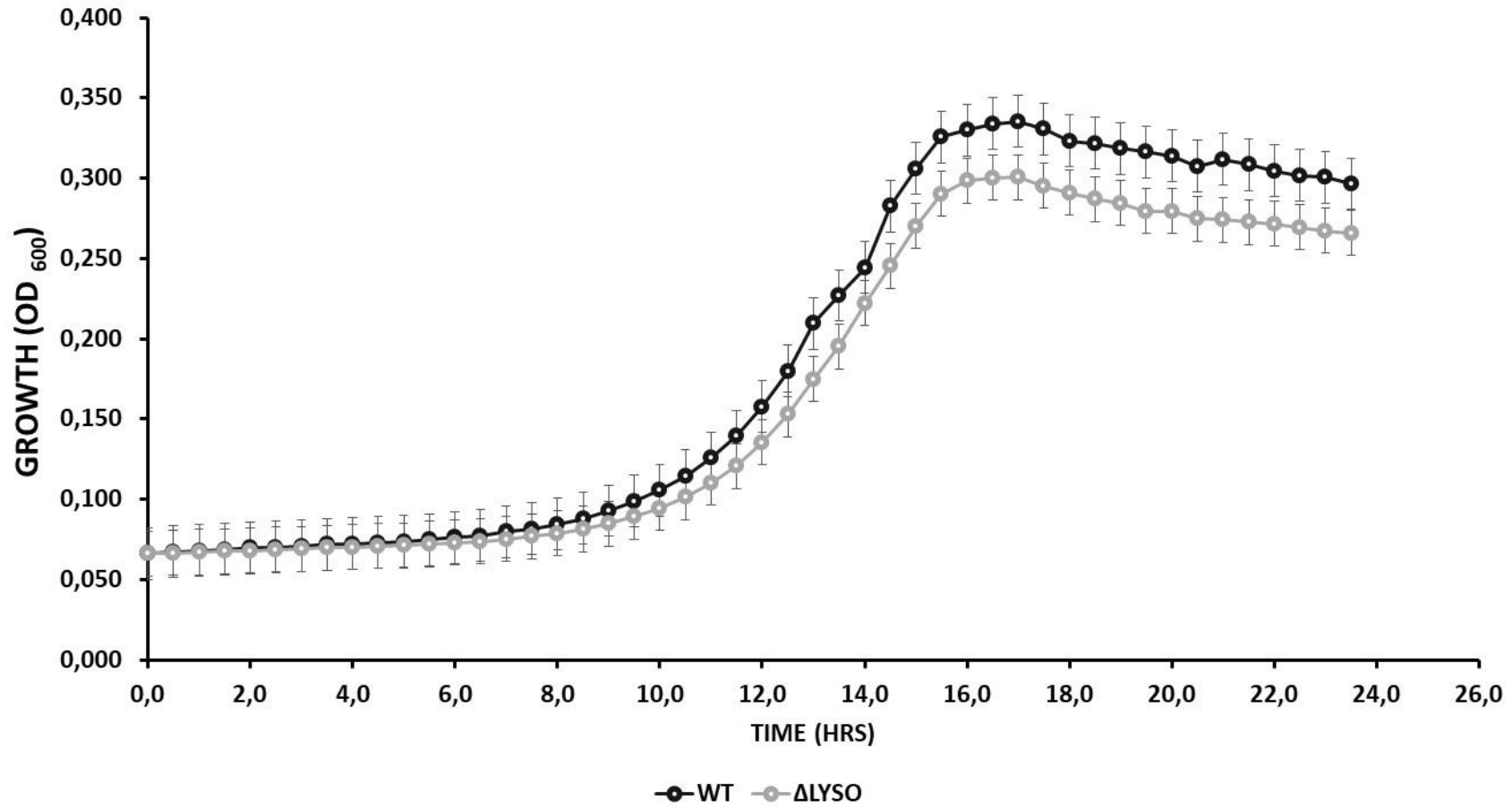
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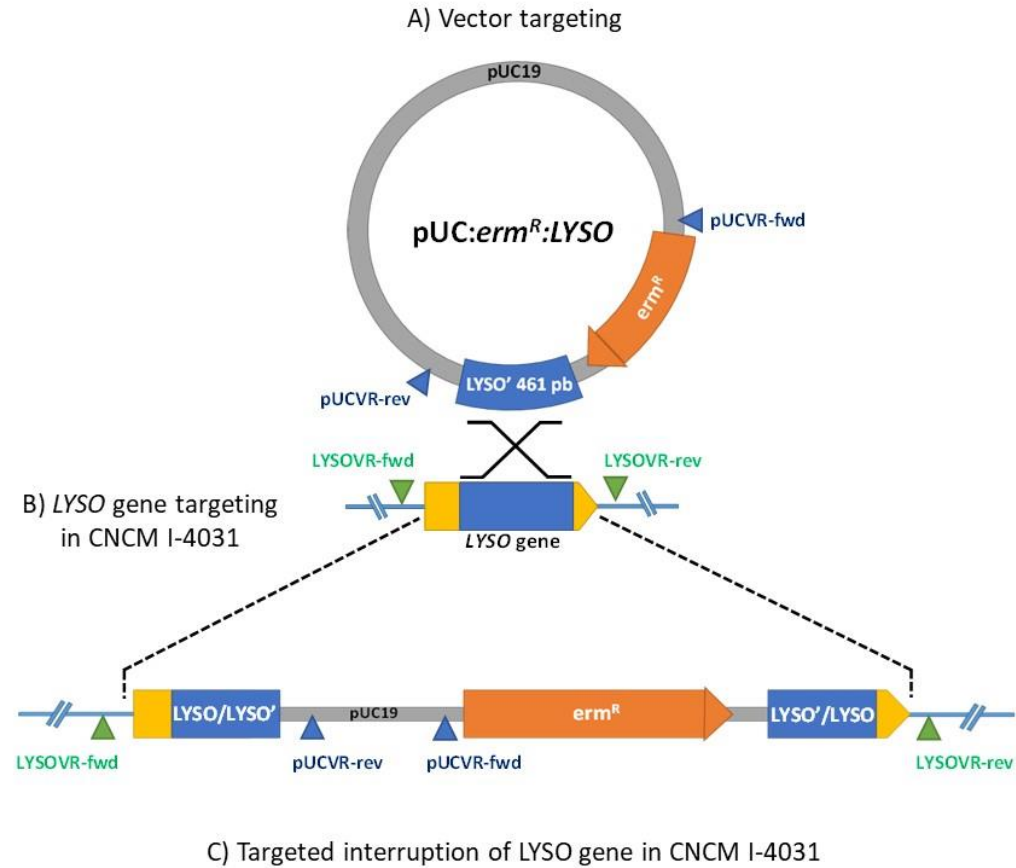
**Supplementary figure S1.** Venn diagram illustrating protein identification of *L. piscium* CNCM I-4031 overlaps among the three biological replicates (R1–R3) in coculture (A) and monoculture (B) conditions, (C) overlap of protein identified in at least two out of the three biological experiments between the both culture conditions and some of the mutually exclusively identified proteins. The total numbers of proteins are indicated for each condition outside the diagram, and the numbers of proteins exclusively detected in each condition or shared between them are indicated within the diagram.



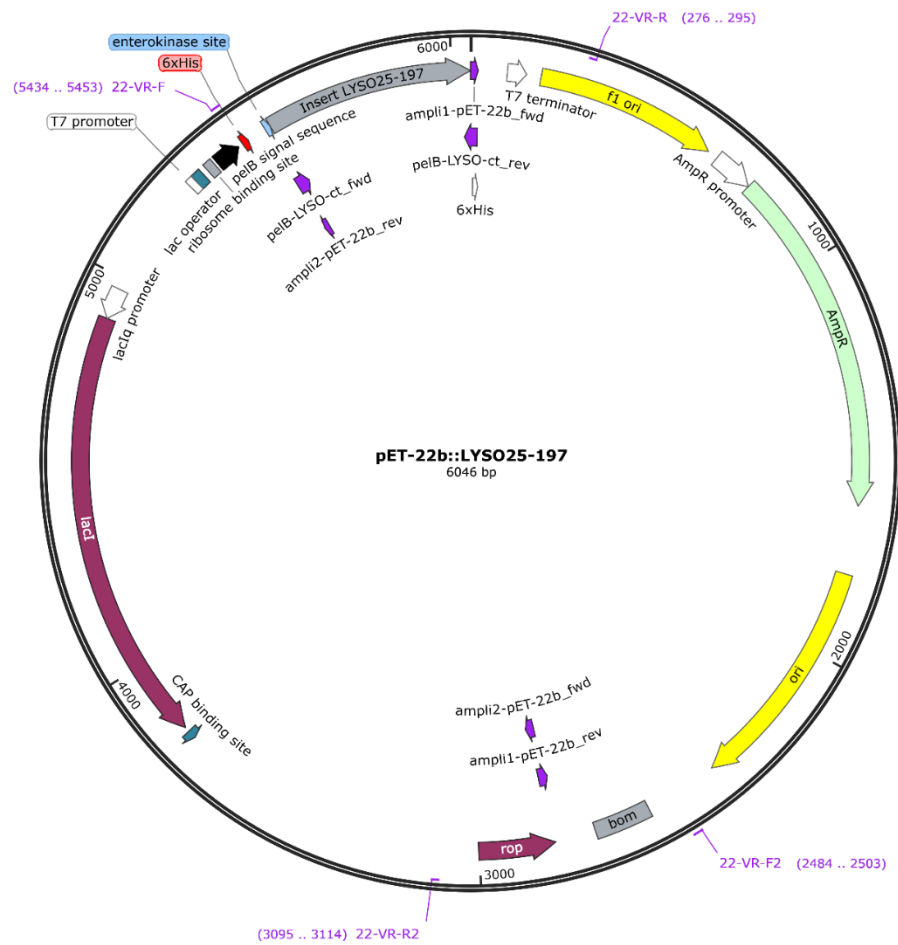
**Supplementary figure S2.** Pearson correlation coefficient values of biological replicates for coculture (A) and monoculture (B) conditions, demonstrating reproducibility. Hierarchical clustering analysis of non-normalized spectral counts for identified *L. piscium* CNCM I-4031 proteins from both coculture and monoculture conditions utilizing a correlation matrix (C).



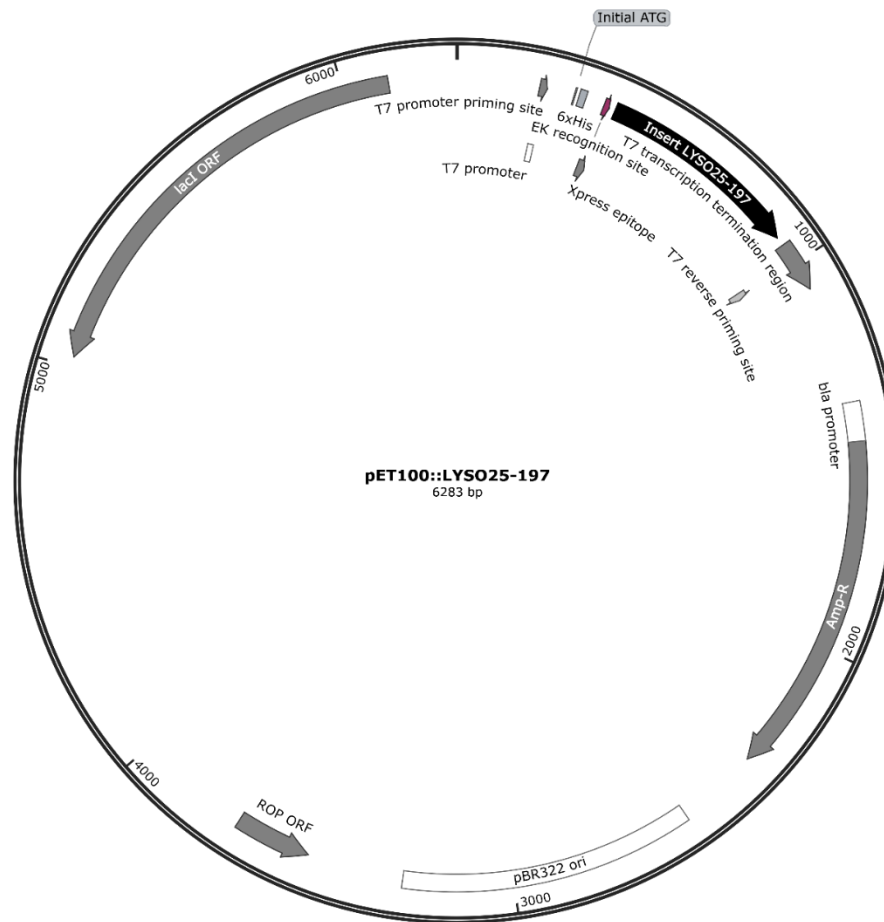
**Supplementary figure S3.** The growth curves of the CNCM I-4031 wild-type strain (WT) and the isogenic  $\Delta$ LYSO strain in MSMA medium at 26°C.



**Supplementary figure S4.** Interruption of *LYSO* gene using suicide vector pUC:*erm<sup>R</sup>*:*LYSO*. (A–C) Schematic view of homologous recombination producing a mutant *L. piscium* CNCM I-4031 $\Delta$ *LYSO*. Disruption of *LYSO* gene in CNCM I-4031 wild-type (WT) by suicide vector pUC:*erm<sup>R</sup>* harbouring 461 bp of *LYSO*. The mutant strain shows resistance to erythromycin by inserting a cassette containing *erm<sup>R</sup>*. The primers for sequencing the insert and characterizing CNCM I-4031 WT and  $\Delta$ *LYSO* are indicated as a blue or green triangle.



**Supplementary figure S5.** pET100 plasmid carrying the sequence encoding for truncated LYSO protein (residues between 25aa -197aa) that lacks the native signal peptide.



**Supplementary figure S6.** pET-22b plasmid carrying the sequence encoding for truncated LYSO protein (residues between 25aa -197aa) that lacks the native signal peptide.

**Supplementary Table S1. Summary of the 863 *L. piscium* CNCM I-4031 proteins present only in all three datasets.** The proteins are grouped according to predicted localization.

Predicted localization		Number of identified proteins	Number in the <i>L. piscium</i> CNCM I-4031 genome	Percent identified	
Cytoplasmic proteins		<b>693</b>	<b>1577</b>	<b>44%</b>	
Cell envelope proteins	Membrane	<b>85</b>	<b>414</b>	<b>21%</b>	
	Surfaceome	LPXTG anchored	<b>2</b>	<b>8</b>	<b>25%</b>
		Lipid anchored	<b>27</b>	<b>49</b>	<b>55%</b>
		N-terminally anchored (No CS)	<b>39</b>	<b>108</b>	<b>37%</b>
		N-terminally anchored (with CS)	<b>7</b>	<b>19</b>	<b>37%</b>
		C-terminally anchored (with CS)	<b>0</b>	<b>1</b>	
		Secretory (released) (with CS)	<b>10</b>	<b>29</b>	<b>34%</b>
	Total surfaceome	<b>85</b>	<b>214</b>	<b>40%</b>	
Total of cell envelope proteins		<b>170</b>	<b>628</b>	<b>27%</b>	
Total proteome		<b>863</b>	<b>2205</b>	<b>39%</b>	



**Supplementary Table S2:** Strains, plasmids and primers used in this study.

<b>Strains</b>	<b>Description</b>	<b>Reference</b>
<i>Lactococcus piscium</i> CNCM I-4031	Bioprotective Strain for Seafood Products	1
<i>Listeria monocytogenes</i> ScottA	Clinical Isolate from a Food-Borne Listeriosis Outbreak	2
<i>E. coli</i> 5-alpha	Derivative of the popular DH5 $\alpha$ . It is T1 phage resistant and endA deficient for high-quality plasmid preparations	NEB
<i>E. coli</i> Lemo21(DE3)	Tunable T7 Expression Strain for difficult targets: membrane proteins, toxic proteins and proteins prone to insoluble expression	NEB
<b>Plasmids</b>		
<b>Plasmids</b>	<b>Description</b>	<b>Reference</b>
pHSP02	Plasmid carrying erythromycin resistance gene (Erm <sup>R</sup> )	3
pUC19	Standard <i>E. coli</i> vector with a multiple cloning site (MCS) for DNA cloning	NEB
pET100	Protein expression vector with an ampicillin resistance marker, for inducible expression of an N-terminally 6xHis-tagged protein	Invitrogen <sup>TM</sup>
pET-22b(+)	Protein expression vectors that encodes a signal sequence for inducible expression of proteins in the periplasm	Novagen
pUC: <i>erm</i> <sup>R</sup> : <i>LYSO</i>	pUC19 carrying erythromycin resistance gene and a 461 bp internal fragment of <i>LYSO</i> gene of strain CNCM-I 4031	This study
pET100:: <i>LYSO</i> <sub>25-197</sub>	pET100 carrying the sequence encoding for truncated <i>LYSO</i> protein (residues between 25aa -197aa) that lacks the native signal peptide	This study / ThermoFisher Scientific
pET-22b:: <i>LYSO</i> <sub>25-197</sub>	pET-22b carrying the sequence encoding for truncated <i>LYSO</i> protein (residues between 25aa -197aa) that lacks the native signal peptide	This study
<b>Primers</b>		
<b>Primers</b>	<b>Sequence (5' to 3')</b>	<b>Reference</b>
<b>Knockout mutants</b>		
Erm <sup>R</sup> gene	fwd: aaacgacggccagtGGGCCCTAGTTTAGAAAAAG rev: ATAAGAGCGCTAGGGACC	This study
<i>LYSO</i> - internal fragment (full-length 461 nt)	fwd: tcctagcgccttatTCCAGCGGACTTCTTTTG rev: tgaccatgattacgccaCGATGACTTTGTGACCCAAAC	This study
pUCVR: primers for sequencing the insert and characterizing wild-type and <i>LYSO</i> mutant	fwd: AGGGTTTTCCCAGTCACGAC rev: TTAGGCACCCCAGGCTTTAC	This study
LYSOVR: Primers for characterizing wild-type and <i>LYSO</i> mutant	fwd: TAGCGGATATAGCCCACAGC rev: TGAAGATGCGGAAACTGTCG	This study

<b>Gene expression in pET22b</b>		
<i>ampli1-pET22b</i>	fwd: ACTCGAGCACCACCACCAC rev: aaacctetgaCACATGCAGCTCCCCGGAG	This study
<i>ampli2-pET-22b</i>	fwd: gctgcatgtTCAGAGGTTTTACCGTC rev: CTTGTCGTCGTCGTCATAC	This study
<i>pelB-LYSO<sub>25-197</sub></i>	fwd: tgtatgacgacgacgacaagGAAAACGTGAAAAGCGTG rev: ggtggtggtgctcaggtTTACCAGTTAAACAGCTG	This study
22-VR1 : primers for sequencing the insert	fwd: CATGGGAGGGTCACATCACC fwd: TGGCGAGAAAGGAAGGGAAG	This study
22-VR2 : Primers for characterizing plasmid	fwd: TACCGCCTTTGAGTGAGCTG fwd: TCCAGTAACCGGGCATGTTC	This study
<b>RNA expression analysis by RT-qPCR</b>		
<i>rpoB</i> gene	fwd: CGAGTTTGTAGATGGTGC GG rev: AAGGGCCATTTCTCCACCTT	This study
<i>RecA</i> gene	fwd: TTCGGAAACACTTTGGCCTG rev: TGGCTCTGAAACTTTAGGGGT	This study
<i>PLY</i> gene	fwd: TTCGGAAACACTTTGGCCTG rev: ACAGCAAGTGCTGACGAAGT	This study
<i>LYSO</i> gene	fwd: GGACTGGTGTGCAAGCCTAT rev: GGC GCAACAACATCTTTGGA	This study

1. Saraoui, T. *et al.* Inhibition mechanism of *Listeria monocytogenes* by a bioprotective bacteria *Lactococcus piscium* CNCM I-4031. *Food Microbiology* **53**, 70–78 (2016).
2. Briers, Y., Klumpp, J., Schuppler, M. & Loessner, M. J. Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. *Journal of Bacteriology* **193**, 4284–4285 (2011).
3. Huang, C. *et al.* CRISPR-Cas9-assisted native end-joining editing offers a simple strategy for efficient genetic engineering in *Escherichia coli*. *Applied Microbiology and Biotechnology* **103**, 8497–8509 (2019).