Supplementary Materials

The multicopy sRNA LhrC controls expression of the oligopeptide-binding protein OppA in *Listeria monocytogenes*

Susanne Sievers[a,b,#], Anja Lund[a,#], Pilar Menendez-Gil[a], Aaraby Nielsen[a], Maria Storm Mollerup[a], Stine Lambert Nielsen[a], Pernille Buch Larsson[a], Jonas Borch-Jensen[a], Jörgen Johansson[c] and Birgitte Haahr Kallipolitis[a,*]

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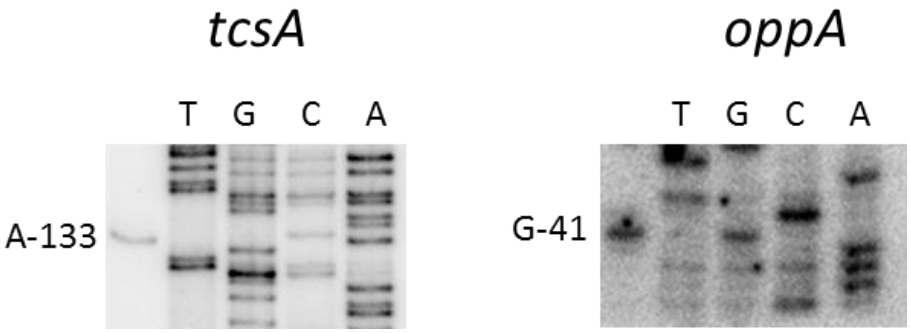
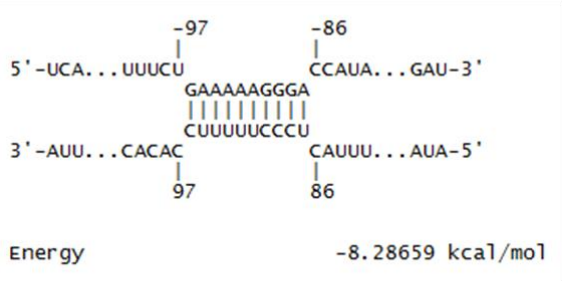


Supplementary Figure S1. Primer extension analysis of *tcsA* and *oppA*. The transcriptional start sites were mapped to -133 and -41, respectively, relative to the translational start-sites (+1).

LhrC1-*tcsA mRNA*



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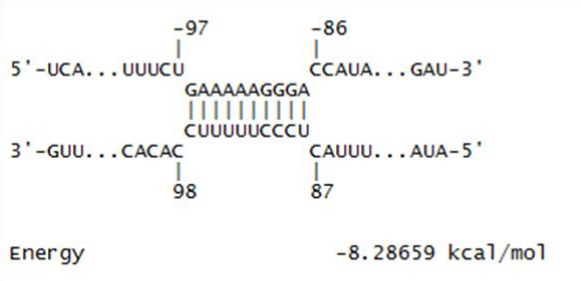

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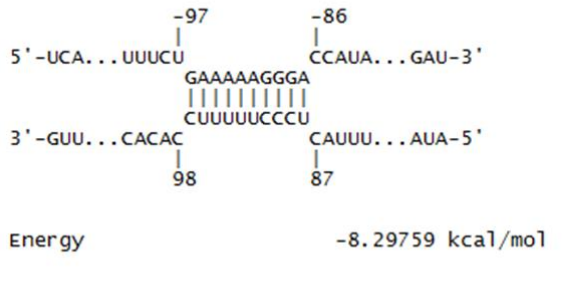

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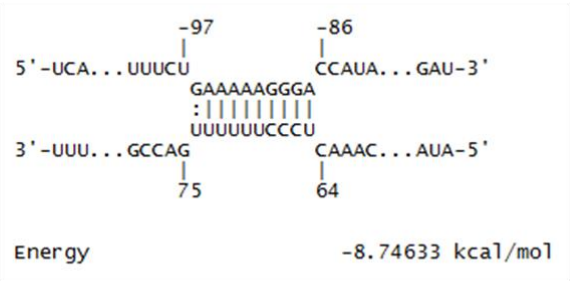
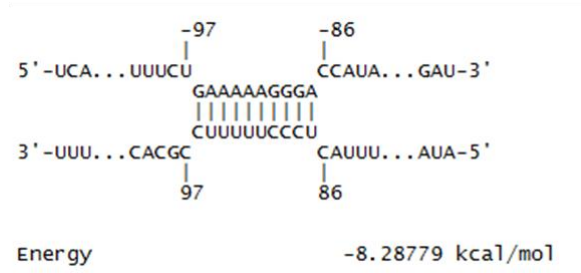
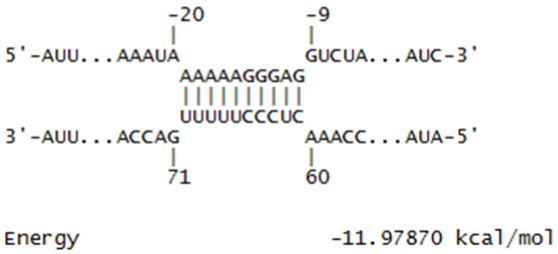


LhrC5-*tcsA mRNA*

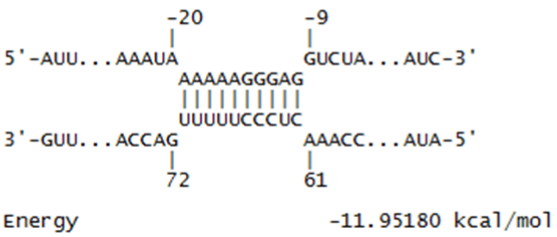


Supplementary Figure S2. Predicted interactions between LhrC1-5 and *tcsA* mRNA. The *tcsA* mRNA sequence is shown on top, whereas sequences for LhrC1-5 are shown below. For *tcsA* mRNA, an AG-rich region far upstream from the translational start-site (+1) is predicted to interact with LhrC1-5. For LhrC1-3 and LhrC5, the predicted interactions involve the CU-rich terminator loop, whereas for LhrC4, the single-stranded stretch is predicted to bind *tcsA* mRNA.

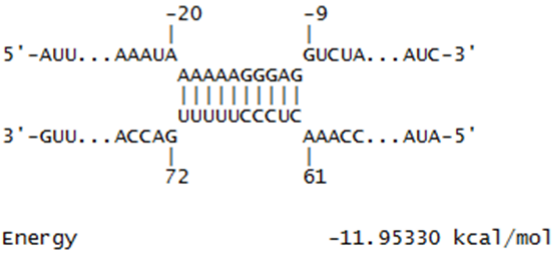
LhrC1-*oppA mRNA*



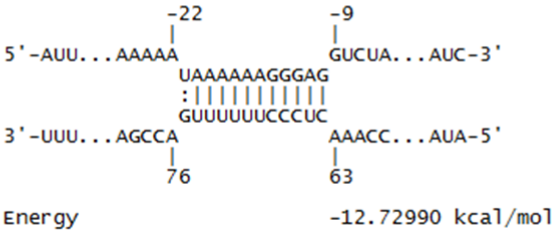
LhrC2-*oppA mRNA*



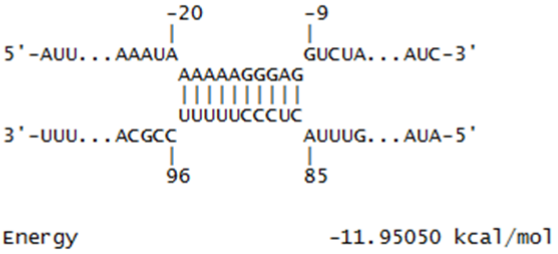
LhrC3-*oppA mRNA*



LhrC4-*oppA mRNA*



LhrC5-*oppA mRNA*



Supplementary Figure S3. Predicted sRNA-mRNA interactions between LhrC1-5 and *oppA* mRNA. The *oppA* mRNA sequence is shown on top, whereas sequences for LhrC1-5 are shown below. For *oppA* mRNA, an AG-rich region right upstream from the translational start-site (+1) is predicted to interact with LhrC1-5. For LhrC1-4, the predicted interactions involve the CU-rich single-stranded stretch, whereas for LhrC5, the terminator loop is predicted to bind *oppA* mRNA.

Supplementary Table S2. RT-qPCR results of *L. monocytogenes* Δ*lhrC1-5* + cefuroxime *vs*. wild type + cefuroxime in comparison with microarray data. Replicate A and B originate from two biologically independent experiments each performed in technical duplicates. Microarray data are the average of three independent experiments.

Gene	Replicate A	Replicate B	Average A+B	Microarray
lmo2210	2.37	2.11	**2.24**	**1.56**
lmo0947	12.31	14.96	**13.64**	**3.07**
lmo0948	3.69	2.74	**3.22**	**2.39**
lmo1388 (tcsA)	3.69	1.53	**2.61**	**1.50**
lmo1378 (lisK)	5.43	4.91	**5.17**	**1.34**
lmo2124	1.59	1.71	**1.65**	**0.91**
lmo2672	0.52	0.61	**0.57**	**0.72**
lmo1299 (glnA)	0.72	0.62	**0.67**	**0.71**
lmo0961	0.60	0.86	**0.73**	**0.71**
lmo1329 (ribC)	0.72	1.03	**0.87**	**1.09**

Supplementary Table S4. Primers used in this study.

Name	Sequence (5´→ 3´)	Further information
NB probes		
F_probe_tcsA_NB	GACCGTTCGTTTAACCAATCAG	Agarose NB, tcsA, forward primer
R_probe_tcsA_NB	GTAGTCTGCTTCAGAAGCTG	Agarose NB, tcsA, reverse primer
F_probe_2196_NB	GAGATATCGTAGCTGGCAAG	Agarose NB, oppA, forward primer
R_probe_2196_NB	GGAATGCGAATAGTGAGTTAATG	Agarose NB, oppA, reverse primer
lhrC_probe	AAAAGGAGTTGGATTTTCATTCT	Agarose NB. Single stranded probe for LhrC.
16S agarose NB F	GTGCATTAGCTAGTTGGTAG	Agarose NB, 16S, forward primer
16S agarose NB R	CAACAGTACTTTACGATCCG	Agarose NB, 16S, reverser primer
transcriptional and translational fusions		
EcoRI_F_lmo2351_promotor	GGGGAATTCTCTGCTGCGCGTTGAAAAC	Forward primer, transcriptional fusion of lmo2351 promoter in pTCV-lac.
BamHI_R2_lmo2351_promoter	CCCCGGATCCTATAAACTTACTTGGTTTTTGG	Reverse primer, transcriptional fusion of lmo2351 promoter in pTCV-lac
EcoRI_F_plhrA_tcsA	GGGGGAATTCCTTGCTTTTTTCAAGAACAATAGTAAAATAAGTTACAGTTGGAGAAGACGTTAAATG	Forward primer, translational fusion of LhrA core promoter + *tcsA* (from -134 to +53 relative to translational start) in pCK-lac
BamHI_R_tcsA_promotor	CCCCGGATCCACGCCAGAAGCAATAATCATTG	Reverse primer, translational fusion of LhrA core promoter + *tcsA* (from -134 to +53 relative to translational start) in pCK-lac
EcoRI_FW_plhrA_2196	GGGGGAATTCCTTGCTTTTTTCAAGAACAATAGTAAAATAAGTTATAGTATCGAAACAATTTTTCAGAA	Forward primer, translational fusion of LhrA core promoter + *oppA* (from -46 to +32 relative to translational start) in pCK-lac
BamHI_R_2196	CCCCGGATCCCCAAGTGTAAGAAATAATTTAG	Reverse primer, translational fusion of LhrA core promoter + oppA (from -46 to +32 relative to translational start) in pCK-lac
Primer		

extensions		
F_Primerex_tcsA	GAGGATTGCAACTAAACTTGATTG	Forward primer, *tcsA* sequencing ladder
Rev_BamHI_tcsA	CCCCGGATCCGATAGTGCTAAAG CAAATG	Reverse primer, sequencing ladder. 5´-end mapping of *tcsA* transcript
F lmo2196 til PE	CATTTCTTCACCTCTAATAAAATTC	5´-end mapping of *oppA* transcript
R lmo2196 til PE	GTGTAAGAAATAATTTAGATTTTTT CAC	Reverse primer, sequencing ladder. 5´-end mapping of *oppA* transcript
RT-qPCR		
lmo0947_F	TCAGCAATTACTTGTTGGAAT	Forward primer
lmo0947_R	CATAGATAATGATAGCGATGGATA A	Reverse primer
lmo0948_F	AATATAGACAACAATGCCAACT	Forward primer
lmo0948_R	GACATACATATCTAACAACAAATC G	Reverse primer
lmo2210_F	TTAATGATAGGGAAGATGAGAAAC	Forward primer
lmo2210_R	TGATAGGTGCTGCTGTAAT	Reverse primer
lmo1388_tscA_F	GCTTCTGGCGTTATCCTA	Forward primer
lmo1388_tscA_R	TCTGTAACCATTGCTACTGTA	Reverse primer
lmo1378_lisK_F	TGTTAGCGGTACAGATAAGT	Forward primer
lmo1378_lisK_R	TTATAGGAAGTGAGCGGATT	Reverse primer
lmo2124_F	GGAGCAGGACATTAGGTAT	Forward primer
lmo2124_R	ATAGTAATTCCAATAGCGATAGC	Reverse primer
lmo1329_ribC_F	ATGGCGAGTATTGGCTAT	Forward primer
lmo1329_ribC_R	TTCTGCTTCTTCTCCGTAA	Reverse primer
lmo0961_F	AGAAGGTGCTGGTGAATT	Forward primer
lmo0961_R	TGTGGATAAGTGTACTGGTAA	Reverse primer
lmo2672_F	GGACTTGACTGGTAGCAT	Forward primer
lmo2672_R	CATTTCAGCAAACCTCTCTT	Reverse primer
lmo1299-F	TTCTTAGCAGGGATGTTGA	Forward primer
lmo1299_R	CTGACCACGCAATGTAAC	Reverse primer
tpi_fw	AACACGGCATGACACCAATC	Forward primer
tpi_rev	CACGGATTTGACCACGTACC	Reverse primer
rpoB_fw	CGTCGTCTTCGTTCTGTTGG	Forward primer
rpoB_rev	GTTCACGAACCACACGTTCC	Reverse primer
EMSA and structural probing		
FW_lmo2196_in_vitro	GGGGTAATACGACTCACTATAGG GAAACAATTTTTCAGAAAAAT	Forward primer, synthesis of oppA DNA with T7 promoter to be transcribed into RNA; also for

		structural probing.
Reverse_lmo2196_in_vitro	CAAGACTAAGCTTAGTAATA	Reverse primer, synthesis of oppA DNA with T7 promoter to be transcribed into RNA; also for structural probing.
T7 _fw_tcsA	GGGGTAATACGACTCACTATAGGGAGTTGGAGAAGACGTTAAATGTTT	Forward primer, synthesis of tcsA DNA with T7 promoter to be transcribed into RNA.
rev_tcsA_gelskift	GATAGTGCTAAAGCAAATGTACG	Reverse primer, synthesis of tcsA DNA with T7 promoter to be transcribed into RNA.
T7_lhrC4_fw	GGGGGAATTCTAATACGACTCACTATAGGGATAAGCTAACAACAAACAAAACATTTTCATTCTTCTCCCCCCTTTTAGAATGAAAATCCC	Forward primer, synthesis of lhrC4 DNA with T7 promoter to be transcribed into RNA; also for structure probing; PCR with overlapping primers (no template)
T7_lhrC4_rev	GGGGGGATCCAAAAAAACCGATGCGGAAAAGGGAGTAAACCGCATCGGTCAAAAAAGGGAGTTTGGGATTTTCATTCTAAAAGGGG	Reverse primer, synthesis of lhrC4 DNA with T7 promoter to be transcribed into RNA; also for structure probing; PCR with overlapping primers (no template)
lhrC4_mut_2_fw	GGGGGAATTCTAATACGACTCACTATAGGGATAAGCTAACAACAAACAAAACATTTTCATTCTTCTCCCCCCTTTTAGAATGAAAATAGAACAG	Forward primer, use with overlapping lhrC4_mut_2_rev primer
lhrC4_mut_2_rev	GGGGGGATCCAAAAAAACCGATGCGGAAAAGGGAGTAAACCGCATCGGTCGGGGTTCCTTCTGTTCTATTTTCATTCTAAAAG	Reverse primer, use with overlapping lhrC4_mut_2_fw primer
lhrC4_mut_3_fw	GGGGGAATTCTAATACGACTCACTATAGGGATAAGCTAACAACAAACAAAACATTTTCATTCTTCTCCCCCCTTTTAGAATGAAAATCCCAAAC	Forward primer, use with overlapping lhrC4_mut_3_rev primer
lhrC4_mut_3_rev	GGGGGGATCCAAAAAAACCGATGCGGTTTTCCCTCTAGTCCGCATCGGTCAAAAAAGGGAGTTTGGGATTTTCATTCTAAAAG	Reverse primer, use with overlapping lhrC4_mut_3_fw primer
lhrC4_mut_4_fw	GGGGGAATTCTAATACGACTCACTATAGGGATAAGCTAACAACAAACAAAACATTTTCATTCTAATGGCGGGAAAAAGAATGAAAATCCCAAAC	Forward primer, use with overlapping lhrC4_mut_4_rev primer
lhrC4_mut_4_rev	GGGGGGATCCAAAAAAACCGATG	Reverse primer, use with

	CGGAAAAGGGAGTAAACCGCATC GGTCAAAAAAGGGAGTTTGGGATT TTCATTCTTTTTC	overlapping lhrC4_mut_4_fw primer
lhrC4_mut_5_fw	GGGGGAATTCTAATACGACTCACT ATAGGGATAAGCTAACAACAAACA AAACATTTTCATTCTTCTCCCCCCT TTTAGAATGAAAATAGAACAG	Forward primer, use with overlapping lhrC4_mut_5_rev primer
lhrC4_mut_5_rev	GGGGGGATCCAAAAAAACCGATG CGGTTTTCCCTCTAGTCCGCATCG GTCGGGGTTCCTTCTGTTCTATTT TCATTCTAAAAG	Reverse primer, use with overlapping lhrC4_mut_5_fw primer
lhrC4_mut_7_fw	GGGGGAATTCTAATACGACTCACT ATAGGGATAAGCTAACAACAAACA AAACATTTTCATTCTAATGGCGGG AAAAAGAATGAAAATAGAACAG	Forward primer, use with overlapping lhrC4_mut_7_rev primer
lhrC4_mut_7_rev	GGGGGGATCCAAAAAAACCGATG CGGTTTTCCCTCTAGTCCGCATCG GTCGGGGTTCCTTCTGTTCTATTT TCATTCTTTTTC	Reverse primer, use with overlapping lhrC4_mut_7_fw primer
Rev_LhrC4_ss_mut	AAAAAAACCGATGCGGAAAAGGG AGTAAACCGCATCGGTCAAAAAAC GCTGTTTGGGATTTTCATTCTAAAA GGGG	Reverse primer for making LhrC4_ss_mut, use in combination with T7_lhrC4_fw.
Rev_LhrC4_ter_mut	AAAAAAACCGATGCGGAAAACGCT GTAAACCGCATCGGTCAAAAAAGG GAGTTTGGGATTTTCATTCTAAAA GGGG	Reverse primer for making LhrC4_ter_mut, use in combination with T7_lhrC4_fw
Rev_LhrC4_combi_mut	AAAAAAACCGATGCGGAAAACGCT GTAAACCGCATCGGTCAAAAAACG CTGTTTGGGATTTTCATTCTAAAAG GGG	Reverse primer for making LhrC4_combi_mut, use in combination with T7_lhrC4_fw
T7_Fw_lmo2196_mut_overlapping	GGGGTAATACGACTCACTATAGG GAAACAATTTTTCAGAAAAATAAAA AACGCTGGTCTACTTAGTGAAAAA ATC	Forward primer, use with overlapping Rev_lmo2196_mut_overlapping
Rev_lmo2196_mut_overlapping	CAAGACTAAGCTTAGTAATAGTGT TAATCCAAGTGTAAGAAATAATTTA GATTTTTTCACTAAGTAGA	Reverse primer, use with overlapping T7_Fw_lmo2196_mut_overlapping