

## Supplementary material for “MprF of *Listeria monocytogenes*”

Table S1: Bacterial strains, plasmids and primers

Plasmids, strains or primers	Genotype or description or sequence	Resistance marker	Reference
<b>Plasmids</b>			
pCR-Blunt II-TOPO	Shuttle vector	Kanamycin (Kan)	Invitrogen
pAUL-A	Shuttle vector, thermosensitive	Erythro-mycin (Em)	(Chakraborty <i>et al.</i> , 1992)
pAUL-A:: $\Delta lmo1695$ pPL2::lmo1695	Shuttle vector with flanked <i>lmo1695</i> gene regions integration vector with <i>lmo1695</i>	Em Chloramphenicol (Cm)	This study
<b>Strains</b>			
<i>E. coli</i> INV $\alpha$ F'	F' <i>endA1 recA1 hsdR17</i> (rk-, mk+) <i>supE44 thi-1 gyrA96 relA1</i> $\phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ ( <i>lacZYA-argF</i> )U169 $\lambda$ -		Invitrogen
<i>Listeria monocytogenes</i> EGD-e	Virulent wild-type, clinical isolate, serovar 1/2a		(Glaser <i>et al.</i> , 2001)
$\Delta lmo1695$	EGD-e deleted in <i>lmo1695</i>		This study
$\Delta prfA$	EGD-e deleted in <i>prfA</i>		(Domann <i>et al.</i> , 1992)
$\Delta lmo1695::lmo1695$	<i>lmo1695</i> complemented with pPL2:: <i>lmo1695</i>		This study
<b>Primers</b>			
A	GGTAATGGCAGACAATGG		This study
B	CGTTAAGGAGACTCTGTTAATCAGAATCTGGTTTTT		This study
C	AAAAACCAGATTCTGATTAACAGAGTCTCCTAACG		This study
D	TTGTTATGTCCACTGTCT		This study
E	ATGTCGGATCCAAAAGCTGATGAGTAACCA		This study
F	ATGTCGCTCGAGAAGAGCAGCTGTAAAGGCTG		This study
G	CGATTAGCCGGACTTGTA		This study
H	GGACAACCTTTACGAGCAG		This study
I	TGGTCATCATGGGCGAAC		This study
J	CATCGTATGTCTGGCAGGT		This study
K	TACGGCTCTGGGTTATTG		This study
L	ATGTTTCGCGATTATCGG		This study
M	GGATTACGAATACCTT		This study
N	ATACTAAACAGTAAATCG		This study
O	TAGCGACGGATAGTCAATC		This study
P	AGACAGTGGACATAACAA		This study
Q	CGCCATGTTAGTTAGTAT		This study

Figure S1

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1          10         20         30         40         50         60
Lmo1695 L.m.  MKEKLMQAYAWFQKNSTVVKIVFITFVMAFVIFELINIATGIDYPSLKENLTSQSPEQIF 60
Mprf_S.aureus  -----MNQEVKKNKIFSILKIFATALFIFVAITLYRELSGINFKDILVEFSKINRMSLV 54
...          : * : : * * * * * : : * * : : : * : : : : * : : : : : * : : : :

          1          10         20         30         40         50

Lmo1695 L.m.  IMFIVGLIAVTPMLLYDVIVKLLPGKFSPSHVIASGWITNTFTNIGGFGGVLGASLRAS 120
Mprf_S.aureus  LFIFGGGASLVLSMYDVILSRALKMDISLSGKVLRVSYIINALNAIVGFGGFIGAGVRAM 114
: * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          60         70         80         90         100        110

Lmo1695 L.m.  FYG-KNASHKEILLAISKIALFLVSGLSIYCLVSLATLLIPGFADHFVNYWPLLAGGLY 179
Mprf_S.aureus  VYKNYTHDKKLVHFISLLILSMLTGLSLLSLLIVFHVFDASLILDKITWVRVVLYVVSF 174
. * : : * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : :

          120        130        140        150        160        170

Lmo1695 L.m.  180      190      200      210      220      230
Mprf_S.aureus  FPILEFITTKWKSLSFVDLPKRELTLIIASLLEWGFAGGCFALIGTLMGEPVDIFKVFP 239
FLPLFIIYSMVRPDKNNRFVG--LYCTLVSCVENLAAAVLVYFCGVIVDAHVSFMSFIA 232
* * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          180        190        200        210        220        230

Lmo1695 L.m.  240      250      260      270      280      290
Mprf_S.aureus  LFVIASVIGIASMPPGGVGTFVDVVMILGSLQSGVSELALAWMLFYRIFYIIPFVVGLL 299
IFIIALSGLVSFIPGGFGADLVLVLGFKTLGVPEEKVLLMLLLYRFAYFVPVIIALI 292
: * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          240        250        260        270        280        290

Lmo1695 L.m.  300      310      320      330      340      350
Mprf_S.aureus  FFVQKAGKKNDNFLEGLPLLFQKVAHRFLVIFVYG-----SGLLLILSSAVPNAIY 351
LSSFFGTSAKKYIEGSKYFIPAKDVTSFLMSYQKDIIAKIPSLSLAILVFFTSMIFFVN 352
: : * : : * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : :

          300        310        320        330        340        350

Lmo1695 L.m.  360      370      380      390      400
Mprf_S.aureus  HVPFLYKIMPFNLETSOITVAFG--FLLLGLARGIECKTKKAYIITVIVLGCAIFN 408
NLTIVYDALYDGNHLYYILLAIHTSACLLLLLVNVGIYKQSRRAIIFAMISILLITVAT 412
: : : * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          360        370        380        390        400        410

Lmo1695 L.m.  410      420      430      440      450      460
Mprf_S.aureus  LARVFSMQAIFLGIVLLCLFLARNEFYREKLVYTWSKVIIDSIIFIVCLAGYIVIGIYN 468
FTYASYILLTWLAIEIVLLIVAFRRARRLKRP-VRMRNVAMLLSFLLVYVNHFFIAG 471
: * : : * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          420        430        440        450        460        470

Lmo1695 L.m.  470      480      490      500      510      520
Mprf_S.aureus  SPNIKHSKEIPDYLRIASEHLLVGFVGVFIAVSVLVIIYYLTSTTEKLGSPFEAVKVR 528
TLYALDIYTIEMHTSVLRYYFWLT----LILIAIIGMIAWLFDYQFSKVRISSKEDECE 527
: : * : : * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : :

          480        490        500        510        520

Lmo1695 L.m.  530      540      550      560      570      580
Mprf_S.aureus  EHLAKWGGNEVSHTMRDRKLLFWAAEGEVLSYRIIADKMVIMCEPTGNMDKMEAAIE 588
EIINQYGGNYLSHLIYSGDKOFFTNENKTAFLMRYKASSIVLVLGDPLGDENAFDELLE 587
* : : * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          530        540        550        560        570        580

Lmo1695 L.m.  590      600      610      620      630      640
Mprf_S.aureus  VMNADRFGYRPVFYEVRGTMIPYLHDHGFDEIKIGERGFVDVONFTMSGKKKGERALM 648
FYNYAEYLGVDVIFYQVTDOHMPLYHNFGNOFFKLGEAIIDLQFSTSGKKRRGFRATL 647
. * : : * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          590        600        610        620        630        640

Lmo1695 L.m.  650      660      670      680      690      700
Mprf_S.aureus  INKEREGVYFEITEPPFNHDWTLRAVSDEIWDGREEKGFSLGFFDTYLEQAIAIAK 708
NKFDELNISFEITEPPFSTEINELQHVSDLWLDNRQEMHFSVGEFNEYLSKAPIGVMR 707
* * : : : * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          650        660        670        680        690        700

Lmo1695 L.m.  710      720      730      740      750      760
Mprf_S.aureus  NGECTIVGFASMPSYTDEMTSIDLMRYSKEAPSGIMDFLFINLFEKAKEDGFQTFNACM 768
NEBEVIAFCSLMPTYFNDAISVDLIRWLPELDLPMDGLYLHMLLWSKEQYTKFNMGM 767
* * : : * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          710        720        730        740        750        760

Lmo1695 L.m.  770      780      790      800      810      820
Mprf_S.aureus  APLANVGESKYAFLGERLAGLVRYSQGFYGFKGLRNFKSKYVTEWEQKFVAFRKSSIA 828
ATLSNVGQLHYSYLRERLAGRVEHEFNGLYRFOGLRRYKSKYNPNWEPRLVRKDNSLW 827
* * * * * : : * * * * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          770        780        790        800        810        820

Lmo1695 L.m.  830      840      850      860
Mprf_S.aureus  FTMLQLMILVGKRPLANSQVVLDFPLEEETKKPDSE 865
ESLSKVMRVIRHK----- 840
: : * * : : * * : : : * * : : : * * : : : * * : : : * * : : : * * : : : * *

          830        840

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Figure S2

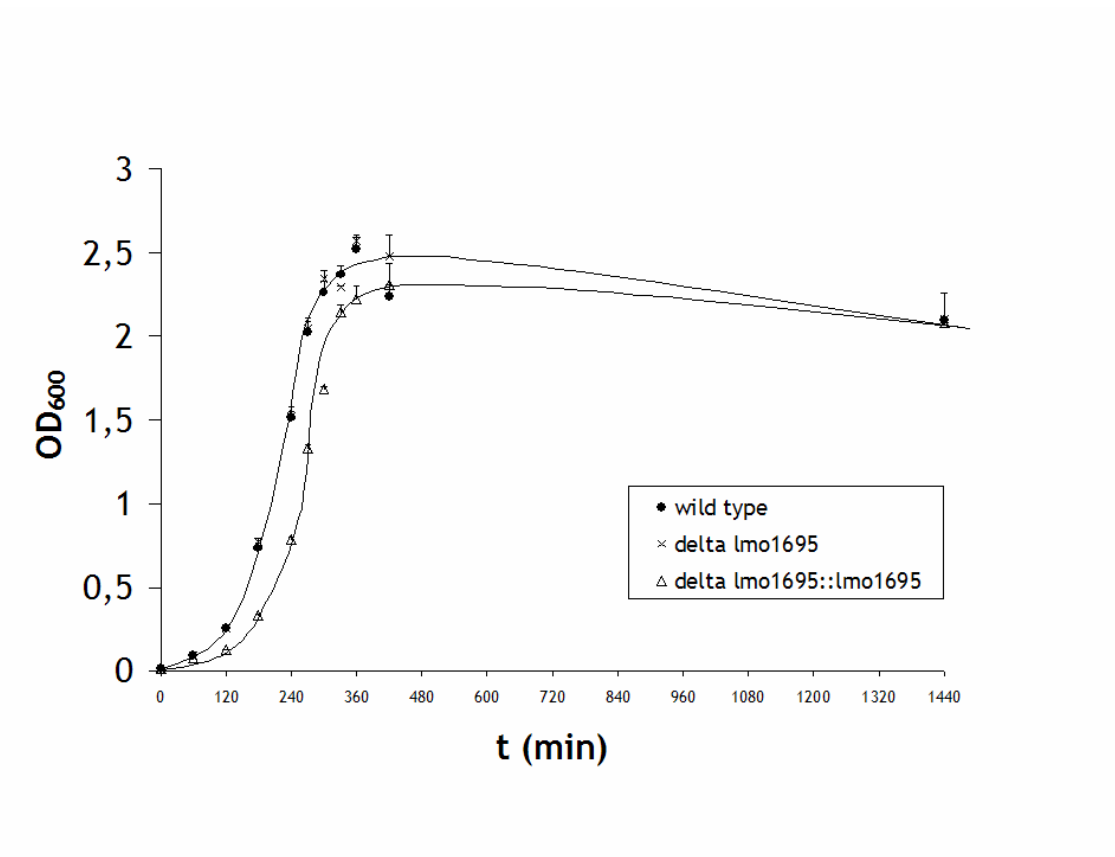


Figure S3

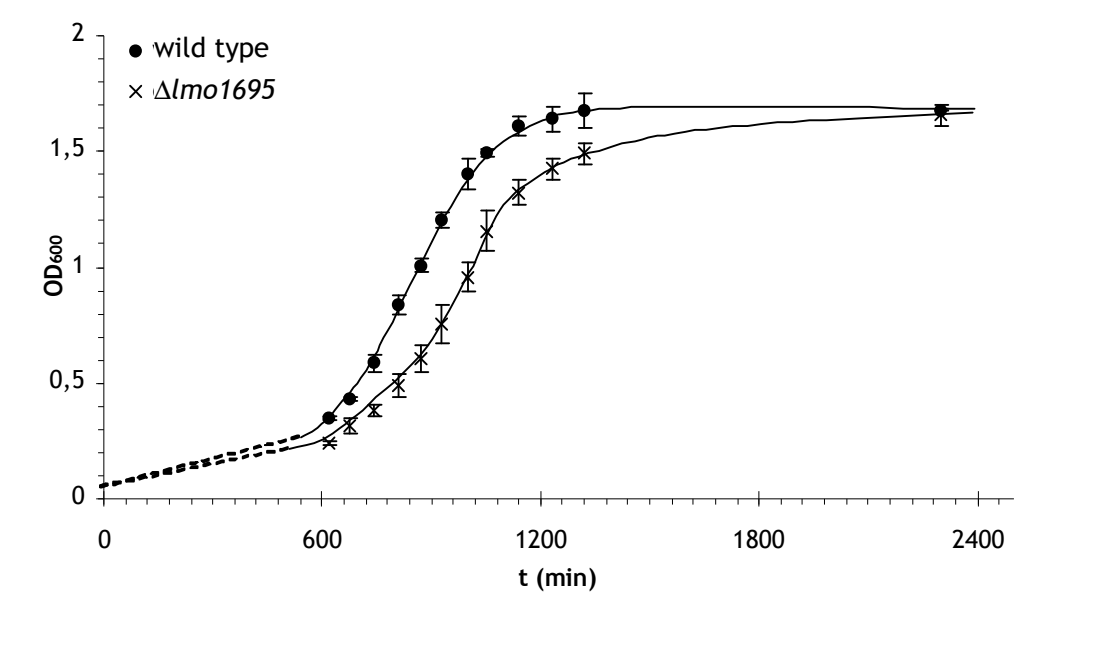


Figure S4

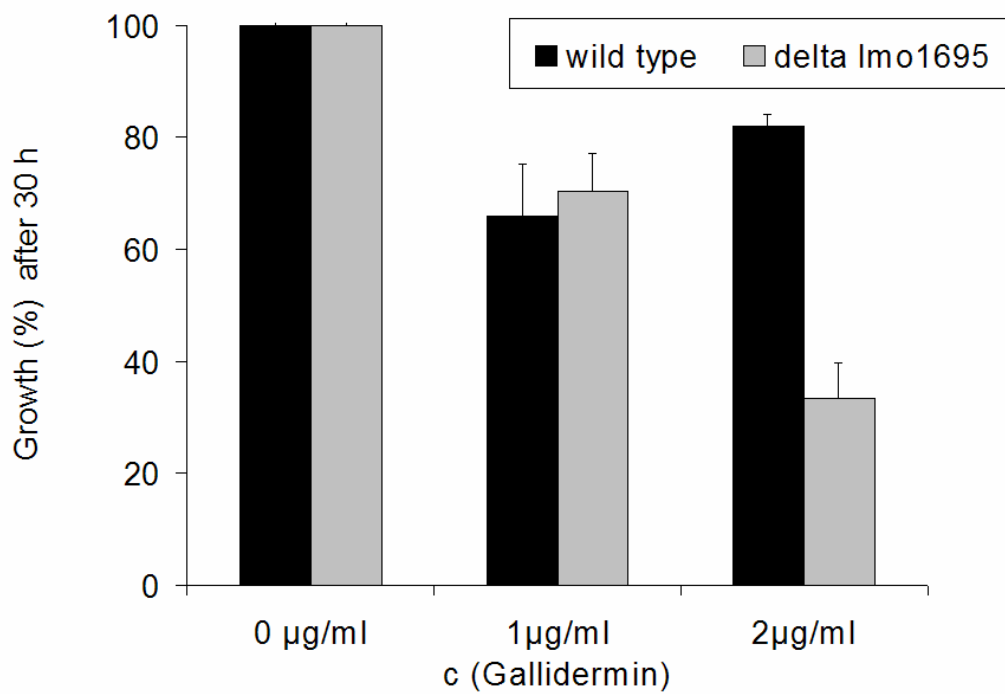
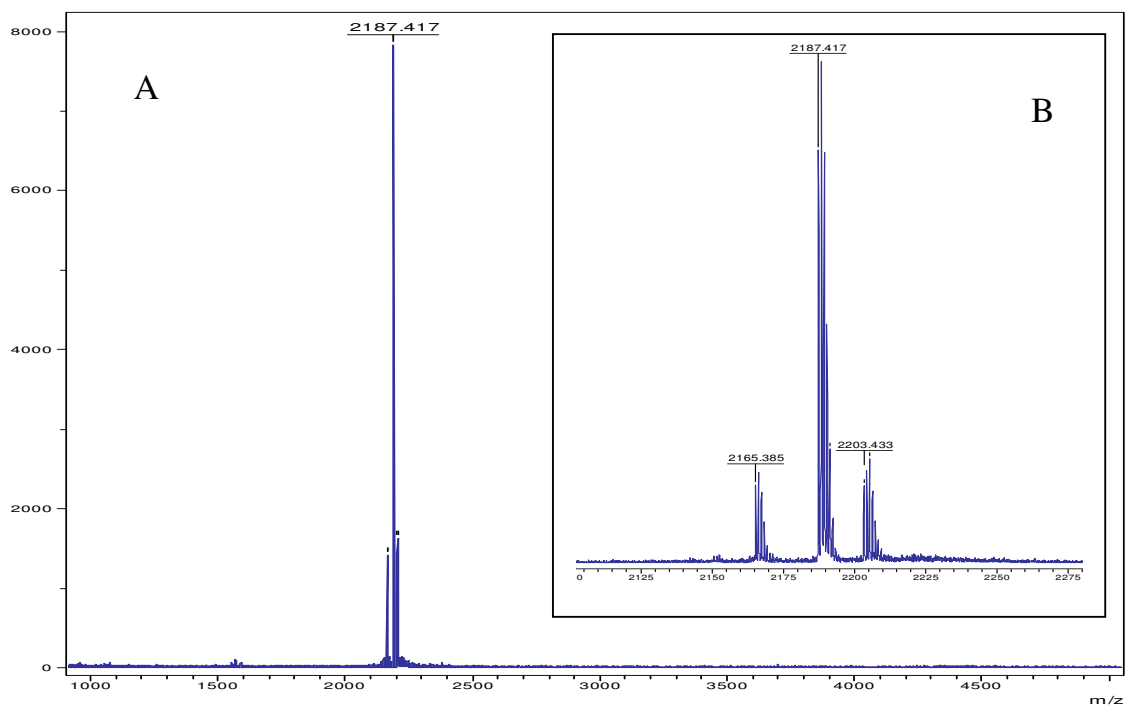


Figure S5



**Fig. S1.** Alignment and comparison of transmembrane domain (TMD) distribution of Lmo1695 (*L. monocytogenes*) and MprF (*S. aureus*). TMDs are underlined and shown in red. Multiple sequence alignment was performed with CLUSTAL W (1.82) (Chenna *et al.*, 2003). Prediction of number, orientation and position of TMDs for Lmo1695 was done using the TopPred2 algorithm, [web.pasteur.fr/seqanal/interfaces/toppred](http://web.pasteur.fr/seqanal/interfaces/toppred) (Claros and von Heijne, 1994). Data for MprF are indicated as by Peschel *et al.* (2001). Peptides which were used to raise polyclonal antibodies are framed. Pfam (Protein families database (Bateman *et al.*, 2004)) domains are highlighted in grey with white fonts (DUF470), black with white fonts (DUF471) and grey with black fonts (DUF472).

**Fig. S2.** Growth curves for all strains used for *in vitro* and *in vivo* infection in BHI medium. The complementation mutant grew slightly slower but reached the same final optical density as the wild type and the deletion mutant.

**Fig. S3.** Growth curves for *L. monocytogenes* wild type and the deletion mutant  $\Delta$ *lmo1695* in minimal medium. Bacteria were cultured in 50 ml minimal medium. The  $\Delta$ *lmo1695* mutant grew slower than the wild type but reached a similar final optical density.

**Fig. S4.** *L. monocytogenes* EGD-e wild type and  $\Delta$ *lmo1695* growth with 0, 1, and 2  $\mu$ g/ml Gallidermin in BHI medium. Values are expressed in %. Growth without Gallidermin was set to 100%. At 1  $\mu$ g/ml Gallidermin and below no significant growth difference was detected. At 2  $\mu$ g/ml the deletion mutant appeared significantly impaired as compared to the wild type. At 4  $\mu$ g/ml Gallidermin growth of both strains was completely inhibited (not shown).

**Fig. S5.** MALDI-MS of gallidermin. Only molecular ions from gallidermin were detected (2165.4 Da, 2187.4 Da and 2203.4 Da [M+H, Na, K]<sup>+</sup> demonstrating the purity of the applied supplement. A: Overview spectrum. B: Detailed view from the molecular ion range.