Supplementary Material, Netterling et al

Supplementary Figure Legends

Supplementary Figure 1 Multiple sequence alignment of Lmo1722 and Lmo1722$_{\Delta CT}$ from *Listeria monocytogenes* and YfmL from *Bacillus subtilis*. Arrow indicates the deletion point of the Lmo1722$_{\Delta CT}$ construct.

Supplementary Figure 2 *L. monocytogenes* and its isogenic Δ*lmo1722* derivative growth on BHI plates at indicated temperatures.

Supplementary Figure 3. Western blot analysis examining expression levels of Lmo1722 in indicated *L. monocytogenes* strains. First 6 lanes show indicated amount of purified Lmo1722. Protein samples of lanes 7-15 were isolated from bacteria grown in BHI at 16°C (except lane 15 where the bacteria were grown at 37°C).

Supplementary Figure 4. Growth of *L. monocytogenes* strains at different temperatures. Indicated bacteria were streaked on BHI-agar plates and incubated at specified temperatures for different length of time.

Supplementary Figure 5. Outline of the primer extension analysis of wild-type + pIMK3 and Δ*lmo1722* + pIMK3 strains as shown in Figure 4C. Fluorescently labeled primers were used

for reverse transcription analysis and the resulting cDNA products were separated and scanned by using a sequencing machine. Purple peaks represent cDNA products and the area of the two most proficient were calculated. The peak representing the mature transcript is an approximately 70 bases long product and the peak representing the immature transcript is an approximately 230 bases long product (bases are shown above graphs (X-axis)). These peaks are indicated by arrows. Absolute fluorescence intensity is shown by the Y-axis.

Supplementary Table 1. Oligonucleotides used in this study

Oligonucleotide name	Sequence 5'-3'	Restriction enzyme sites
lmo1722-A	CCCGTCGACGATGGTAAGGTAATTGTCATCG	*Sal*I
lmo1722-B	CCCGAATTCGATAGATAGGAAAGCATTCGC	*EcoR*I
lmo1722-C	CCCGAATTCCGCTTACTGCAAGTCTGCAC	*EcoR*I
lmo1722-D	CCCCCATGGGATGGATTACTATCGGGAGG	*Nco*I
1722 Dp+/p-	CCCCCCGTCGACTTAAATGGCATCCTCGCAAC	*Sal*I
1722 Up-	CCCCCCGGATCCGAGTCGGGTTATGACAGAATCAA	*BamH*I
degU-F	CGTGAATACCGCCATTTAGC	
degU-R	GAAAAGTGTCTCCCCAATTCC	
flaA-F	CGTGAACAATCAATCCATCG	
flaA-R	TTAACATTTGCGGTGTTTGG	
tmRNA-U	CGGCACTTAATATCTACGAGC	
tmRNA-D	CCTCGTTATCAACGTCAAAGCC	
lmo1722-F_64_KpnI	AATTGGTACCGTGCAGACTTGCAGTAAGCGT	*Kpn*I
lmo1722-RH_66_XbaI	AATTTCTAGATTAGTGATGGTGATGGTGATG AGATTTTTTAAAATTGTTTGGTTTTTTG	*Xba*I
lmo1722-F_64_BamHI	AATTGGATCCGTGCAGACTTGCAGTAAGCGT	*BamH*I
lmo1722-R_62_SalI	AATTGTCGACATGGCATCCTCGCAACGATAA	*Sal*I
lmo1722-R_noC_64_SalI	AATTGTCGACTTAGTTTTCTGGAACAGCACCGTC	*Sal*I

Supplementary Figure 1

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CLUSTAL 2.1 multiple sequence alignment


Lmo1722      MTES-----NIPSFWAEKWQEHGYETPTEIQSAMYQPIKDGADVLAVSPTGTGKTVAYAL 55
YfmL         MTQTWPFLHNAQSFIQENWNASGFQKPTPVQEQAAQLIMDGKDVIAESPTGTGKTLAYAL 60
             **::     *   **  *:*:  *::.** :*.   * * ** **:* ********:****

Lmo1722      PTLEKIEAIPKT-QWLVLAPSHELVMQITEVIRSWLPSDDLTVISLIGGANVKRQIEKLK 114
YfmL         PVLERIKPEQKHPQAVILAPSRELVMQIFQVIQDWKAGSELRAASLIGGANVKKQVEKLK 120
             *.**:*:.   *   * ::****:****** :**:.* ...:* . **********:*:****

Lmo1722      KKPQIIVASPGRALELIKQKKIKMHEIKTITLDECDQLLRQENFKSTLEIVESAVRDRQL 174
YfmL         KHPHIIVGTPGRVFELIKAKKLKMHEVKTIVLDETDQLVLPEHRETMKQIIKTTLRDRQL 180
             *:*:***.:***.:**** **:*****:***.*** ***:  *: ::  :*:::::***** 

Lmo1722      TLVSATKLENPDMFFAQTENTPVMLEVTAKPEELTNVEHLYMDVESRDKATLLRRISHIK 234
YfmL         LCFSATLKKETEDVLRELAQEPEVLKVQRSKAEAGKVKHQYLICDQRDKVKLLQKLSRLE 240
              .***  :::. .: :  : * :*:*  .   *  :*:* *:  :.***..**:::*::: 

Lmo1722      DMRGLVFVKDKPRMEILLEKLHYDKVKAAGIHGEIRKEKRKKYLDDFKKGTLTYLIVTDV 294
YfmL         GMQALVFVRDIGNLSVYAEKLAYHHVELGVLHSEAKKMERAKIIATFEDGEFPLLLATDI 300
             .*:.*****:*   .:.:   *** *.:*: . :*.* :* :* * :   *:.* :. *:.**: 

Lmo1722      AARGLDIEDLPYVIHYDLAASEKEYTHRSGRTGRMGKSGTVITFANPREIRTLKQYLTIH 354
YfmL         AARGLDIENLPYVIHADIPD-EDGYVHRSGRTGRAGKEGNVLSLVTKLEESKLK------ 353
             ********:****** *:.  *. *.********* **.*.*::::.. *  .** 
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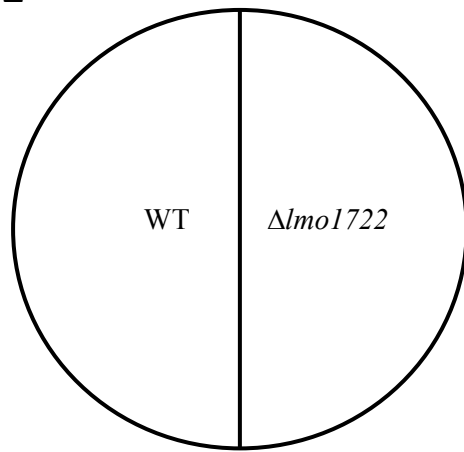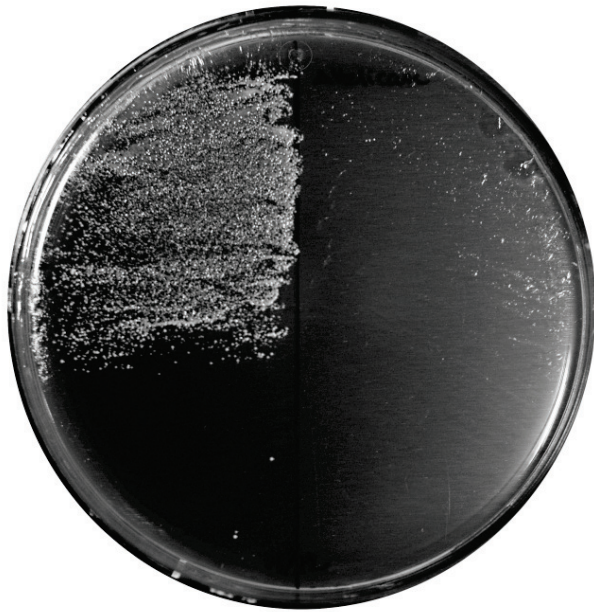
▼ Lmo1722ΔCT

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Lmo1722      NLKGKQVRFYQGKLLDGAVPENKIAKKASRPPKQVQKSKKDDGKKPFRKDKPTGAAGGRK 414
YfmL         ---------------------KMAKKLG-----VELS------------EAVYAGGKLK 374
                                  *:*** .     *: *             :.. *.*  *

Lmo1722      EKPTNSAKPTRNKPNKFNKKPNNFKKS 441
YfmL         TK------------------------ 376
              *
```
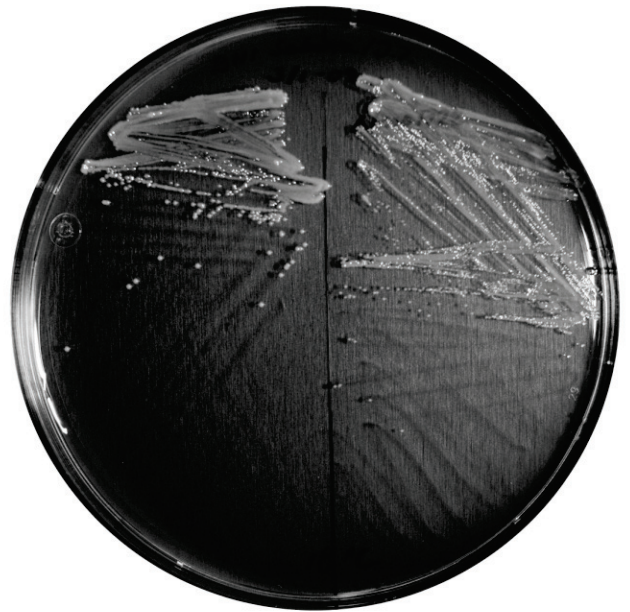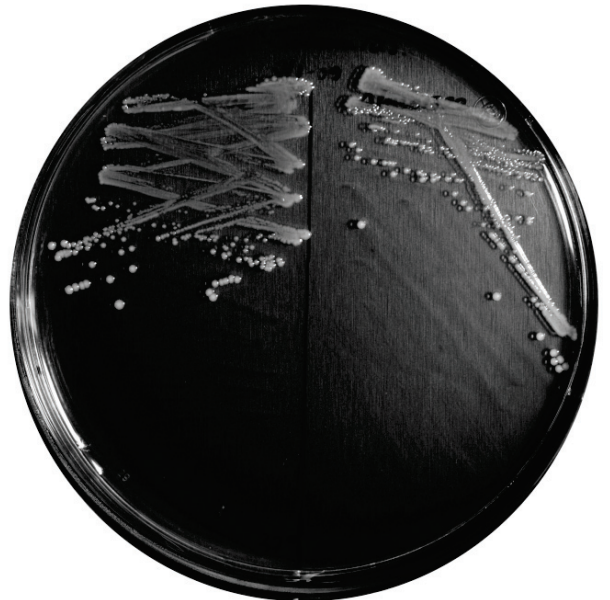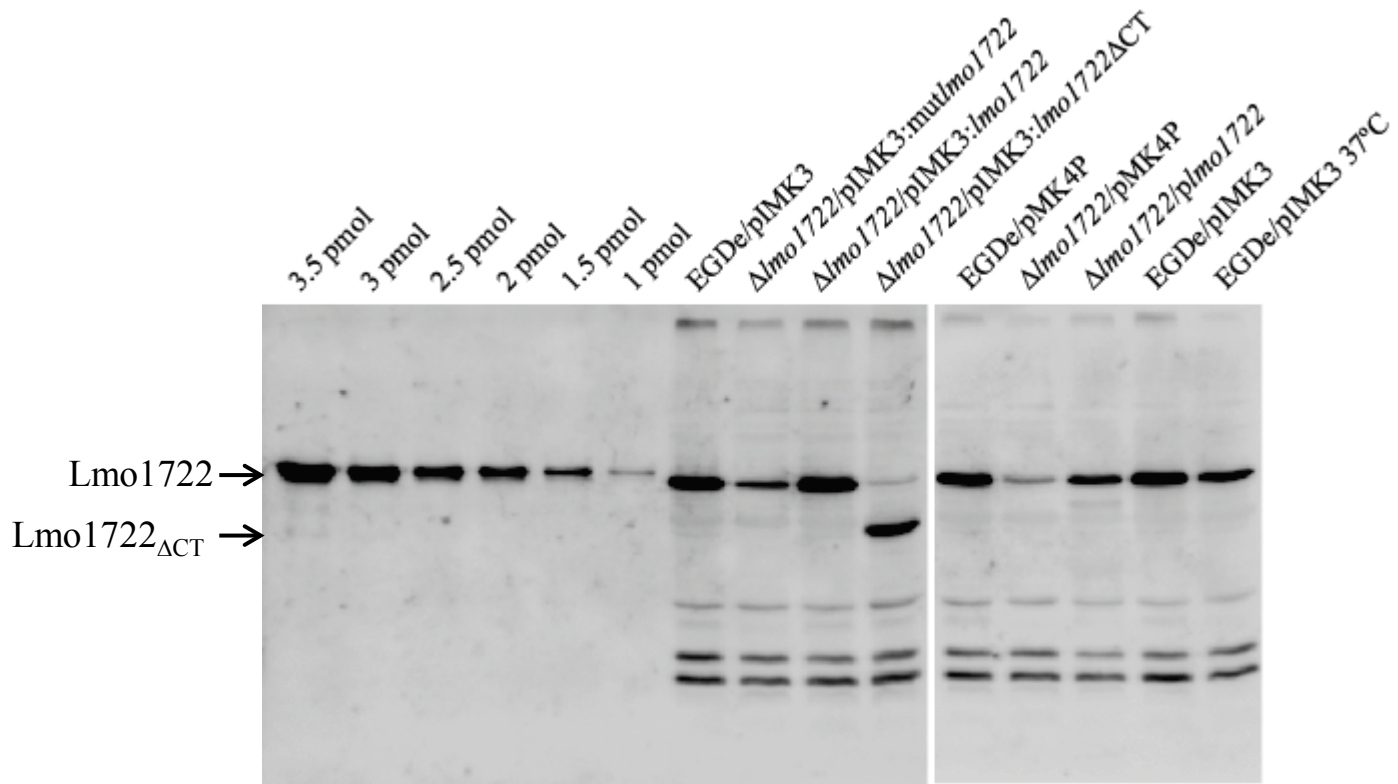
Supplementary Figure 2

Supplementary Figure 3

Lanes: 3.5 pmol, 3 pmol, 2.5 pmol, 2 pmol, 1.5 pmol, 1 pmol, EGDe/pIMK3, $\Delta lmo1722$/pIMK3:mut$lmo1722$, $\Delta lmo1722$/pIMK3:$lmo1722$, $\Delta lmo1722$/pIMK3:$lmo1722\Delta$CT, EGDe/pMK4P, $\Delta lmo1722$/pMK4P, $\Delta lmo1722$/p$lmo1722$, EGDe/pIMK3, EGDe/pIMK3 37°C

Lmo1722 →
Lmo1722$_{\Delta CT}$ →

Supplementary Figure 4

Δ*lmo1722*/
pIMK3:*lmo1722*$_{\Delta CT}$

EGDe/
pIMK3

Δ*lmo1722*/
pIMK3:*lmo1722*

Δ*lmo1722*/
pIMK3

0 ˚C
120 days

5 ˚C
60 days

16 ˚C
4 days

26 ˚C
2 days

37 ˚C
1 day
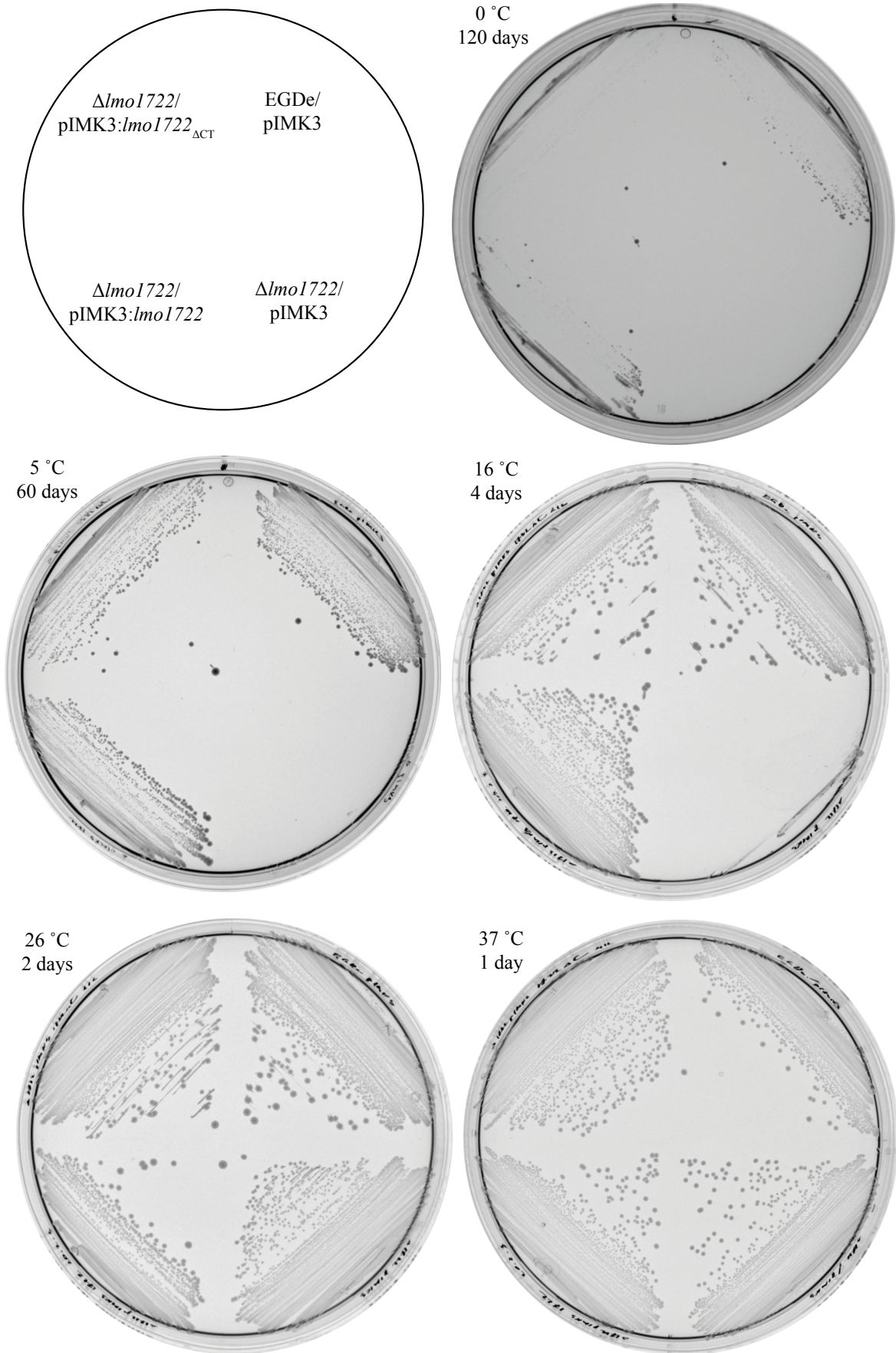
Supplementary Figure 5

EGDe/pIMK3

Bases

Mature 23S rRNA   Immature 23S rRNA

Δ*lmo1722*/pIMK3

Bases

Mature 23S rRNA   Immature 23S rRNA