# *Francisella* is sensitive to Insect Antimicrobial Peptides

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## Supplementary Information

## Methods

*Analysis of LPS phenotypes*

The determination LPS variants followed [1] and [2] with slight modifications. Bacteria were grown in Chamberlain medium overnight and diluted to $OD_{600\ nm}$ 1.0 before lysis. To roughly estimate the amount of material obtained we spectrophotometrically determined the protein concentration (Nanodrop, Saveen & Werner). Aliquots corresponding to 10µg protein were separated in precast 4-12% Novex NuPAGE Bis-Tris gels (1mm thick, Invitrogen) using 1x MES-SDS buffer. Stained carbohydrates were visualized using an Alpha Imager Mini with high UV range (Alpha Innotech).

*Measurement of surface charge*

Bacteria were grown in Chamberlain medium overnight. Pelleted bacteria were resuspended in phosphate buffer (32mM $Na_2HPO_4$, 7mM $KH_2PO_4$, pH 7.4) to $OD_{600nm}$ 1.0. Samples were measured using a Zetasizer Nano ZS particle analyzer (Malvern Instruments); the Smoluchowski approximation was applied to determine zeta potential values.

## References

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3 Åhlund MK, Ryden P, Sjöstedt A, Stöven S: Directed screen of francisella novicida virulence determinants using drosophila melanogaster. Infect Immun 2010;78:3118-3128.
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**Table S1.** Sequence of oligonucleotide primers used for quantitative real-time PCR.

| Gene | forward primer | reverse primer |
|---|---|---|
| *AttA* | 5´-TGACGCACAGCAACTTCC-3´ | 5´-AAGGTAGTAGCACGATTGGG-3´ |
| *CecA* | 5´-TTCGTCGCTCTCATTCTGG-3´ | 5´-CATCCCGAGTGTGCTGAC-3´ |
| *Def* | 5´-GCCAGAAGCGAGCCACAT-3´ | 5´-CGGTGTGGTTCCAGTTCCA-3´ |
| *DptA* | 5´-TTCTACTTTGGCTTATCCGATG-3´ | 5´-TCCATATCCTCCATTCAGTCC-3´ |
| *Drc* | 5´-GCACAATGAAGTTCACCATCGT-3´ | 5´-CCACACCCATGGCAAAAAC-3´ |
| *Drs* | 5´TCAAACAGAAATCATTTACCAAGC3´ | 5´-CCCAGGACCACCAGCATCAG-3´ |
| *rp49* | 5´-TGACCATCCGCCCAGCATACA-3´ | 5´-TCTCGCCGCAGTAAAC-3´ |

**Table S2.** Phenotypic characteristics of selected attenuated *F. novicida* transposon insertion mutants with regard to their surface features and their virulence in *Drosophila melanogaster*[a].

| Locus Tag | Gene name | Surface charge[b] [mV] | LPS phenotype | in wildtype flies: Fly survival[c] ΔMLL [days] | in wildtype flies: *in vivo* prolif.[d] day 2 | in *Relish*$^{E20}$ flies: Fly survival[c] ΔMLL [days] | in *Relish*$^{E20}$ flies: *in vivo* prolif.[d] day 2 |
|---|---|---|---|---|---|---|---|
| cell wall/LPS/capsule | | | | | | | |
| FTN_0071 | *htrB* | -4* | wt-like | | | | |
| FTN_0107 | *lepA* | -3 | wt-like | -0.1 | nd | nd | nd |
| FTN_0713 | *ostA2* | -3 | wt-like | | | | |
| FTN_1174 | *murI* | -3 | wt-like | | | | |
| FTN_1214 | | -2 | wt-like | | | | |
| FTN_1215 | *kpsC* | -1 | wt-like | | | | |
| FTN_1220 | | -3 | wt-like | -0.7 | nd | nd | nd |
| FTN_1222 | *kpsF* | -6* | no O-antigen | 3.0* | 8.3* | 0.3 | 13.3 |
| Motility, attachment and secretion structure | | | | | | | |
| FTN_0415 | *pilA* | -3 | wt-like | | | | |
| FTN_0672 | *secA* | -3 | wt-like | | | | |
| FTN_0861 | *pilA* | -2 | wt-like | | | | |
| FTN_1115 | *pilB* | -3 | wt-like | | | | |
| FTN_1141 | *pilM* | -4 | wt-like | | | | |
| FTN_1692 | | -3 | wt-like | -0.1 | nd | nd | nd |
| (conserved) hypothetical membrane protein/unknown | | | | | | | |
| FTN_0030 | | -2* | wt-like | | | | |
| FTN_0096 | | -3 | wt-like | | | | |
| FTN_0109 | | -3* | wt-like | | | | |
| FTN_0275 | | -2 | wt-like | | | | |
| FTN_0325 | | -3 | wt-like | 0.1 | nd | nd | nd |
| FTN_0354 | *tolA* | -2* | wt-like | 3.5* | 2.7* | 1.7 | 4.7 |
| FTN_0357 | *pal* | -2* | wt-like | 3.8* | 8.9* | 0.6* | 7.2* |
| FTN_0398 | | -3 | wt-like | 2.7 | nd | nd | nd |
| FTN_0444 | | -1 | wt-like | 1.1 | nd | nd | nd |
| FTN_0478 | | -2 | wt-like | -0.5* | 11.2* | nd | nd |
| FTN_0534 | | -3 | wt-like | | | | |
| FTN_0538 | | -3 | wt-like | | | | |
| FTN_0706 | | -3 | wt-like | | | | |
| FTN_0717 | | -2* | wt-like | | | | |
| FTN_0759 | | -3 | wt-like | | | | |
| FTN_0886 | | -3 | wt-like | -0.3 | nd | nd | nd |
| FTN_0888 | | -3 | wt-like | | | | |
| FTN_1038 | | -2* | wt-like | | | | |
| FTN_1223 | | -3 | wt-like | | | | |
| FTN_1257 | | -2 | wt-like | | | | |
| FTN_1406 | | -2* | wt-like | -0.8 | nd | nd | nd |
| FTN_1582 | | -3 | wt-like | | | | |

[a] Median values are given throughout; *, $P<0.05$ for mutant versus U112 comparison; [#], $P<0.05$ for comparison of effects in *Relish*$^{E20}$ versus wildtype flies. Mutant strains for analysis were reported as attenuated in virulence or in proliferation in flies in at least one of three studies [3-5]; strains listed had a significantly lower surface charge than the U112 wildtype in a first round of zeta potential measurements. Genes were grouped according to the genome annotation in [6].

[b] The surface charge was measured as zeta potential, the average of 3 measurements á 100 runs is given. The zeta potential of the U112 wildtype strain was -2.8mV.

[c] Fly survival is given as difference in median life length (ΔMLL) between mutant– and U112-infected flies. Results are from 3 experiments á 20-30 flies. For comparison, the median MLL of U112-infected wildtype flies was 4.6+/-0.2 days (n=6) and that of U112-infected *Relish*$^{E20}$ flies was 2.2+/-0.1 days (n=3).

[d] Bacterial proliferation in flies is given as $\log_2$ CFU/fly and is the absolute increase in bacteria per fly from injection (day 0) to day 2 post infection. For comparison, the proliferation of U112 in wildtype flies on day 2 post infection was 8.1 $\log_2$ CFU/fly (n=2-3) and 10.1 $\log_2$ CFU/fly in *Relish*$^{E20}$ flies (n=3).

**Table S3.** Effects of *F. novicida* transposon insertions in known LPS biosynthesis genes on fly survival and bacterial proliferation in flies[a]

| Gene name | Locus tag | Surface charge[b] [mV] | LPS phenotype | in wildtype flies: Fly survival[c] ΔMLL [days] | in wildtype flies: Prolif. index[d] day 2 | in *Relish*$^{E20}$ flies: Fly survival[c] ΔMLL [days] | in *Relish*$^{E20}$ flies: Prolif. index[d] day 2 |
|---|---|---|---|---|---|---|---|
| O antigen | | | | | | | |
| ----- | *FTN1256* | -14* | no O-antigen | -1.0 | 2.1* | *nd* | *nd* |
| *wbtH* | *FTN1421* | -17* | no O-antigen | 0.0 | -0.9 | *nd* | *nd* |
| *wbtG* | *FTN1423* | -17* | no O-antigen | 0.0 | -0.9 | *nd* | *nd* |
| *wbtF* | *FTN1425* | -14* | no O-antigen | 0.6 | 0.8 | *nd* | *nd* |
| *wbtE* | *FTN1426* | -15* | no O-antigen | 0.2 | -0.1 | *nd* | *nd* |
| *wbtD* | *FTN1427* | -14* | no O-antigen | 0.3 | -0.4 | *nd* | *nd* |
| *wbtO* | *FTN1428* | -11* | no O-antigen | -0.6 | 1.3 | *nd* | *nd* |
| *wbtQ* | *FTN1430* | -15* | no O-antigen | -0.3 | 2.1 | *nd* | *nd* |
| *wbtA* | *FTN1431* | -14* | no O-antigen | -0.8 | 1.1 | *nd* | *nd* |
| Kdo core | | | | | | | |
| *manB* | *FTN1417* | -17* | no O-antigen, smaller core | 0.0 | -2.4* | -0.9*[#] | -1.1 |
| *manC* | *FTN1418* | -26* | no O-antigen, smaller core | -0.2 | -0.5 | *nd* | *nd* |
| lipid A | | | | | | | |
| *lpxF* | *FTN0295* | -5* | reduced O-antigen, smaller core | 9.9* | $-9.6_3$* | 1.1[#] | -6.8* |
| *lpxE* | *FTN0416* | -2 | wt-like | 0.4 | $0.7_3$ | *nd* | *nd* |
| *flmF2* | *FTN0545* | -2 | wt-like | 0.5 | $-0.5_3$ | *nd* | *nd* |
| *flmK* | *FTN0546* | -2 | reduced O-antigen | -0.2 | $0.4_3$ | *nd* | *nd* |
| *flmF1* | *FTN1403* | -2 | wt-like | 0.3 | $-0.8_3$ | *nd* | *nd* |
| cell wall | | | | | | | |
| *slt* | *FTN0496* | -3* | reduced O-antigen | 2.9* | -2.8* | 1.0* | -1.8* |

[a] Median values are given throughout; *, $P<0.05$ for mutant versus U112 comparison; [#], $P<0.05$ for comparison of effects in *Relish*$^{E20}$ versus wildtype flies.

[b] The surface charge was measured as zeta potential, the average of 3 measurements á 100 runs is given. The zeta potential of the U112 wildtype strain was -2.8mV, and that of LVS -1mV.

[c] Fly survival is given as difference in median life length (ΔMLL) between mutant– and U112-infected flies. Results are from 2-3 experiments for wildtype and from 3-4 experiments for *Relish*$^{E20}$ mutant flies; 40-100 flies per experiment. For comparison, the

median MLL of U112-infected wildtype flies was 3.3 +/-0.1 days (n=14) and that of U112-infected *Relish*$^{E20}$ flies was 2.5+/-0.1 days (n=8).

[d] Proliferation index is the difference in *in vivo* proliferation between mutant and wildtype bacteria expressed as $\log_2$ CFU/fly. Negative values indicate that mutant bacteria proliferated slower in flies than the U112 wildtype. Where indicated ($_3$), values for day 3 post infection are given. For comparison the proliferation of U112 in wildtype flies on day 2 post infection was 11.1 $\log_2$ CFU/fly and 13.1 $\log_2$ CFU/fly in *Relish*$^{E20}$ flies.

**Table S4.** Effect of phagocytosis inhibition on the virulence of *lpxF* and *manB* mutant bacteria in wildtype flies [a]

| treatment | Fly survival [b] ΔMLL [days] | Proliferation index [c] day 1 | day 3 |
|---|---|---|---|
| mock + *lpxF* | 7.9 * | -7.4* | -12.1* |
| beads + *lpxF* | 9.3 *[#] | -11.9* | -11.5* |
| mock + *manB* | 0.2 * | -0.9 | *nd* |
| beads + *manB* | -0.3[#] | -2.6 | *nd* |

[a] Median values are given throughout; *, $P<0.05$ for mutant versus U112 comparison; [#], $P<0.05$ for comparison of treatment with beads versus from mock treatment with PBS.

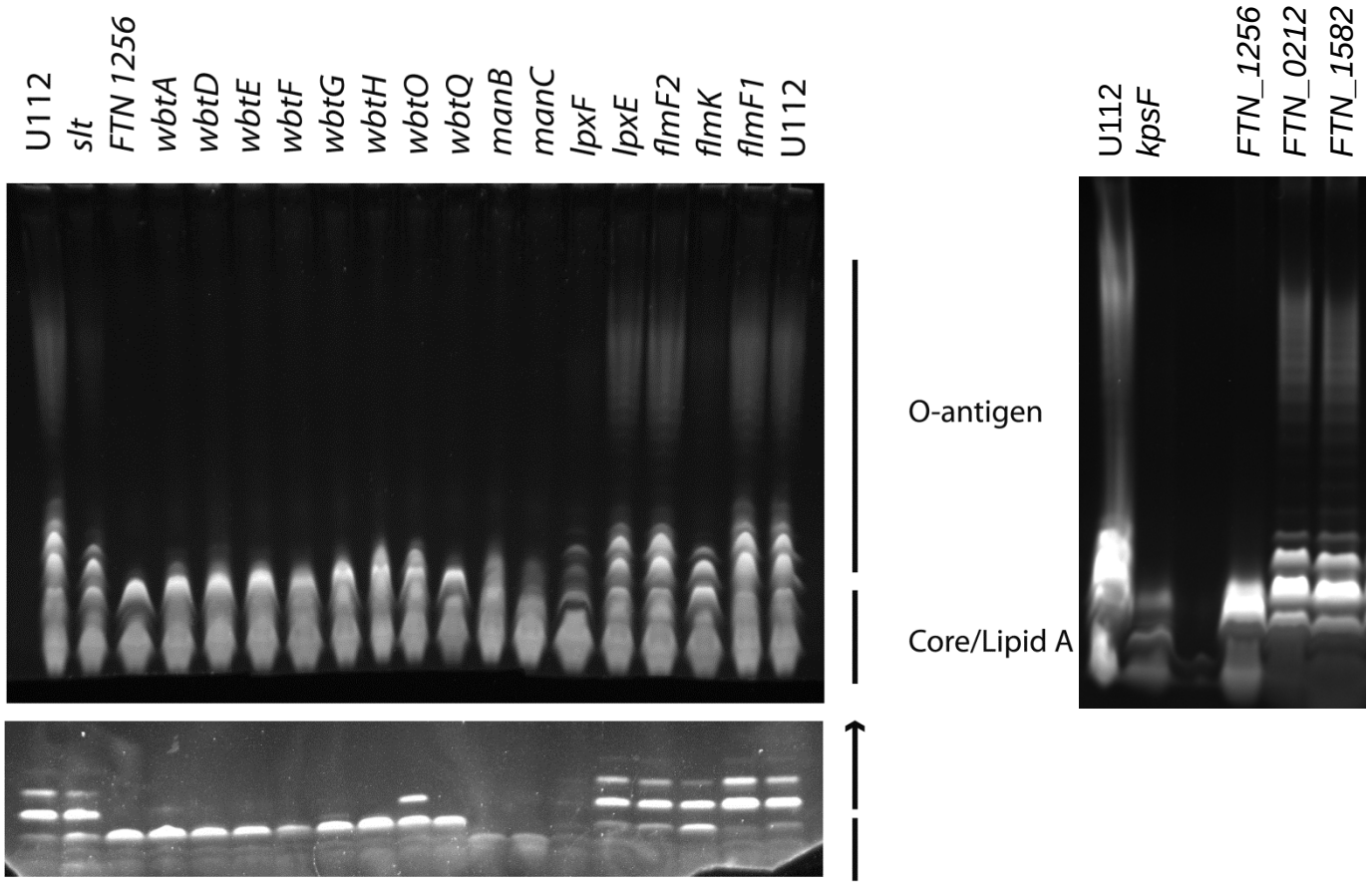
[b] Fly survival is given as difference in median life length (ΔMLL) between mutant– and U112-infected flies. Results are from 2-3 experiments are shown. For comparison, the median MLL of bead-treated U112-infected flies was 3.1+/-0.2 days.

[c] Proliferation index is the difference in *in vivo* proliferation between mutant and wildtype bacteria expressed as $\log_2$ CFU/fly. Negative values indicate that mutant bacteria proliferated slower in flies than the U112 wildtype. For comparison the proliferation of U112 in bead-treated flies on day 1 post infection was 8.7 $\log_2$ CFU/fly.

**Figure S1.** LPS profiles of the *F. novicida* U112 wildtype and U112-derived mutant strains. Crude bacterial extracts were separated by gel electrophoresis and carbohydrates were visualized using a fluorescent dye. To demonstrate the weakly stained O-antigen samples in the upper panels were overloaded with material corresponding to approximately 180μg protein. For the lower panel only approximately 20μg protein was loaded per sample to obtain good separation of core and lipid A components.

**Figure S2.** LPS profiles of *F. novicida* U112 wildtype and U112-derived mutants in *manB, lpxF* and *slt*. Crude bacterial extracts corresponding to the indicated amounts of protein were separated by gel electrophoresis and carbohydrates were visualized using a fluorescent dye. The grayscale picture file was inverted to more clearly visualize LPS components of low abundance.

Vonkavaara et al., Figure S1



Vonkavaara et al., Figure S2