

## SUPPLEMENTAL MATERIAL

### SUPPLEMENTAL RESULTS

**Microarray analysis of *F. tularensis* LVS within hepatocytes:** As described in *Materials and Methods*, the global gene expression of LVS organisms grown in FL83B hepatocytic cells for 48 hours was compared to that of bacteria cultured in broth for the same period using cDNA microarrays. Data averaged from four independent hybridizations showed that expression of 53 genes was up-regulated and that of 20 genes was down-regulated more than two-fold in intracellular bacteria, as compared to bacteria cultured in MH+; these are listed in Table S2 below. The most highly upregulated genes belong to the *fsl* operon and are discussed in the text. Most of the other genes encode hypothetical proteins with unknown functions. Most also are present in the genomes of both the LVS and the highly virulent, type A Schu S4 strain. Twelve of the 17 genes that are located in the *Francisella* pathogenicity island were up-regulated from 2.0- to 5.0-fold, including pathogenicity island gene G (*pigG*), *iglD*, *pigB*, *pigI*, *pigA*, *pigD*, *pdpC*, *pigC*, *pdpA*, *pigH*, *pdpD*, and *pdpB*. Other pathogenicity island genes also were up-regulated but to a lesser extent, including *pigE* (1.8-fold), *iglC* (1.6-fold), *iglB* (1.8-fold), and *iglA* (1.8-fold). Genes encoding MglA and MglB, which regulate expression of virulence genes and are required for growth in macrophages (1-4), were down-regulated approximately 2-fold.

1. **Baron GS, Nano FE.** 1998. MglA and MglB are required for the intramacrophage growth of *Francisella novicida*. *Mol. Microbiol.* **29**:247-259.
2. **Brotcke A, Weiss DS, Kim CC, Chain P, Malfatti S, Garcia E, Monack DM.** 2006. Identification of MglA-regulated genes reveals novel virulence factors in *Francisella tularensis*. *Infect. Immun.* **74**:6642-6655.
3. **Lauriano CM, Barker JR, Yoon SS, Nano FE, Arulanandam BP, Hassett DJ, Klose KE.** 2004. MglA regulates transcription of virulence factors necessary for *Francisella tularensis* intraamoebae and intramacrophage survival. *Proc. Natl. Acad. Sci. U. S. A* **101**:4246-4249.
4. **Santic M, Molmeret M, Klose KE, Jones S, Kwaik YA.** 2005. The *Francisella tularensis* pathogenicity island protein IglC and its regulator MglA are essential for modulating phagosome biogenesis and subsequent bacterial escape into the cytoplasm. *Cell Microbiol.* **7**:969-979.

**Table S1: Primers used in this work**

Primer No.	Sequence <sup>a</sup>
1	5'-GCAAGCTGAGCATATTCTCCCAG-3'
2	5'-CACTTGTAGCATGAATTGCCACTG-3'
3	5'-CGAGATTTACCGCAATGGC-3'
4	5'-GCTTAGAGTAGGGCTTATTCCTGC-3'
5	5'-TGGACCGATACTGACACTGAGG-3'
6	5'-AGAGCCTTTACACCGACTCC-3'
7	5'-AGACgtcgacTGCTTCGCCATGCTTTATCATC-3'
8	5'-AGACcatatgCCAATATGACAATGTAGCCCAGC-3'
9	5'-AGACcatatgTGAGCTAGGACGTAGTTTGGTCG-3'
10	5'-AGACgtcgacCTTGGCTTTAGATGGATGAACTCG-3'
11	5'-CCTATGGAAAAACGCCAGCAAC-3'
12	5'-AATACGCAAACCGCCTCTCC-3'
13	5'-TCGAGATTTACCGCAATGGC-3'
14	5'-TCGCTTAGAGTAGGGCTTATTCCTG-3'
15	5'-TGCTTTTCTCTCCTTTTTGGGG-3'
16	5'-GCTGCTATGATTATCGGGTCTGAG-3'
17	5'-GCCACTATCTGAAATCTATGGTCGC-3'
18	5'-CGAGTCCTTGAACAAAACGCC-3'
19	5'-ggatccGCTCTAGTTGGCAATCCAAACTGC-3'
20	5'-catatgACCAATACCTTTTGCTGCAACGACT-3'
21	5'-catatgACACTTGCTAAAGAGGTTGTAGTCGG-3'
22	5'-gtcgacGCAGCCAGTAAGATTTGCCACG-3'
23	5'-TGATATTGGTAGTCTGTGGCTTGC-3'
24	5'-CATTGCGAGAATACCTGTGACAG-3'
25	5'-AGACcatatgGAAATCTCTGTGGCATTTCCTGG-3'
26	5'-AGACacgcgtGGCAGCAAGAAATATACGCCC-3'
27	5'-AGACacgcgtGCAGATTGTTGAATCATTCGGTGC-3'
28	5'-AGACcatatgTTTACCAAGAAGCCCCTCACG-3'
29	5'-GGAGCCTATGGAAAAACGCC-3'
30	5'-GGAGAGCCTGAACCTTTGAAGC-3'
31	5'-AGTCgtcgacCAGGAACTCAAATCAAGGTATGTAAGG-3'
32	5'-AGTCggatccCGCCTGATTCGCTTTTATTACGACATTTC-3'
33	5'-AGTCgtcgacGAAATCTCTGTGGCATTTCCTGG-3'
34	5'-AGTCggatccGGCAGCAAGAAATATACGCCC-3'

<sup>a</sup>The following restriction sequences are indicated in lower-case letters: Sal I: gtcgac; Nde I: catatg; MluI: acgcgt; BamH I: ggatcc.

**Table S2: Up- and down-regulated genes of the LVS grown in FL83B hepatocytic cells for 48 h in comparison with the LVS grown in MH+ broth**

ORF in LVS <sup>a</sup>	ORF in Schu S4 <sup>a</sup>	Product	Symbol	P-Value	Fold Change
FTL_1834	FTT0027c	diaminopimelate decarboxylase	<i>fslC</i>	4.4E-10	13.1
FTL_1832	FTT0029c	hypothetical protein	<i>fslA</i>	3.8E-08	10.6
FTL_1835	FTT0026c	hypothetical protein	<i>fslD</i>	5.4E-10	9.1
FTL_1833	FTT0028c	hypothetical protein	<i>fslB</i>	5.1E-06	6.7
FTL_1164	FTT1707	hypothetical protein	<i>pigG</i>	3.1E-09	5.0
FTL_0675	FTT1388	hypothetical protein		3.1E-09	4.1
FTL_0953	FTT0677c	hypothetical protein		5.4E-10	3.8
FTL_1218	FTT0981	hypothetical protein		1.2E-06	3.6
FTL_1160	FTT1711c	intracellular growth locus, subunit D	<i>iglD</i>	2.6E-07	3.6
FTL_0816	FTT1140	hypothetical protein		3.2E-10	3.5
FTL_0867	FTT0602c	hypothetical protein		1.4E-06	3.5
FTL_0207	FTT0296	Pyrrolidone-carboxylate peptidase	<i>pcp</i>	1.5E-07	3.5
FTL_1169	FTT1702	hypothetical protein	<i>pigB</i>	2.1E-07	3.4
FTL_0473	FTT0403	peptide deformylase	<i>defI</i>	1.5E-08	3.3
FTL_1161	FTT1710	hypothetical protein	<i>pigI</i>	7.4E-05	3.1
FTL_1170	FTT1701	hypothetical protein	<i>pigA</i>	1.9E-05	3.0
FTL_0026	NA <sup>b</sup>	3-hydroxyisobutyrate dehydrogenase		6.1E-10	3.0
FTL_1217	NA <sup>b</sup>	hypothetical protein		2.3E-05	2.9
FTL_0815	NA <sup>b</sup>	PRC-barrel		3.0E-07	2.9
FTL_1231	FTT0970	hypothetical protein		5.3E-07	2.9
FTL_0449	FTT0383	hypothetical protein		1.2E-06	2.8
FTL_1678	FTT0101	conserved membrane hypothetical protein		7.5E-06	2.8
FTL_1228	FTT0973	hypothetical protein		2.2E-07	2.8
FTL_1167	FTT1704	hypothetical protein	<i>pidD</i>	2.0E-08	2.7
FTL_1162	FTT1709	hypothetical protein	<i>pdpC</i>	2.5E-03	2.7
FTL_1168	FTT1703	conserved hypothetical protein.	<i>pigC</i>	2.6E-06	2.7
NA <sup>b</sup>	FTT1143	hypothetical protein		3.4E-06	2.7
FTL_1219	FTT0980	hypothetical protein		5.0E-04	2.6
FTL_1230	FTT0971	cysteine desulfurase activator complex subunit SufB	<i>sufB</i>	1.2E-06	2.5
FTL_1172	FTT1699	hypothetical protein	<i>pdpA</i>	1.2E-06	2.5
FTL_1163	FTT1708	hypothetical protein	<i>pigH</i>	2.6E-05	2.5
FTL_1154	NA <sup>b</sup>	pseudogene		1.9E-07	2.5
NA <sup>b</sup>	FTT1143	hypothetical protein		4.7E-07	2.4
FTL_1755	FTT0133	glycerol uptake facilitator protein	<i>glpF</i>	1.3E-08	2.4
FTL_1229	FTT0972	ABC transporter, ATP-binding protein	<i>sufC</i>	2.2E-06	2.4

FTL_0924	NA <sup>b</sup>	proton-dependent oligopeptide transporter		1.0E-06	2.4
FTL_1016	FTT0558	short chain dehydrogenase		3.9E-06	2.4
FTL_0663	FTT1400c	hypothetical protein		1.8E-02	2.3
FTL_1503	FTT0720c	deoxyguanosinetriphosphate triphosphohydrolase	<i>dgt</i>	2.3E-05	2.3
FTL_1790	FTT0070c	major facilitator superfamily (MFS) transport protein	<i>ampG</i>	2.2E-04	2.2
FTL_0348	NA <sup>b</sup>	pseudogene		1.2E-06	2.2
FTL_0569	FTT1542c	outer membrane protein		5.8E-04	2.2
FTL_1709	FTT1639c	hypothetical protein		5.4E-07	2.2
FTL_0456	FTT0390c	30S ribosomal protein S21	<i>rpsU</i>	3.4E-03	2.2
FTL_0217	FTT0306	fumarate hydratase, Class II	<i>fumC</i>	2.1E-07	2.1
FTL_0208	FTT0297	hypothetical membrane protein		4.5E-04	2.1
NA <sup>b</sup>	FTT0421	pseudogene		5.7E-04	2.1
FTL_1509	NA <sup>b</sup>	D-alanyl-D-alanine carboxypeptidase/D-alanyl-D-alanine-endopeptidase	<i>dacB2</i>	1.4E-05	2.1
FTL_1603	FTT0461	RNA-binding protein	<i>yhbY</i>	1.9E-06	2.0
FTL_1601	FTT0463	tRNA/rRNA methyltransferase	<i>yibK</i>	2.6E-05	2.0
FTL_1156	FTT1715c	hypothetical protein	<i>pdpD</i>	4.4E-05	2.0
FTL_1171	FTT1700	hypothetical protein	<i>pdpB</i>	1.4E-02	2.0
NA <sup>b</sup>	FTT0852	pseudogene		3.1E-04	2.0
FTL_1184	FTT1276	macrophage growth locus, subunit B	<i>mglB</i>	5.9E-05	-2.0
FTL_0046	FTT1647c	dirooorotate dehydrogenase	<i>pyrD</i>	7.1E-05	-2.0
FTL_0345	FTT0849	bile acid symporter family protein		1.1E-03	-2.1
FTL_0680	FTT0563	polyamine transporter, subunit H, ABC transporter, membrane protein	<i>potH</i>	1.0E-03	-2.1
FTL_0075	FTT1674	riboflavin synthase beta subunit (6,7-dimethyl-8-ribityllumazine synthase)	<i>ribH</i>	1.2E-06	-2.1
FTL_0261	FTT0350	DNA-directed RNA polymerase, alpha subunit	<i>rpoA2</i>	1.8E-04	-2.1
FTL_0245	FTT0334	30S ribosomal protein S17	<i>rpsQ</i>	1.6E-03	-2.1
FTL_1185	FTT1275	macrophage growth locus, subunit A	<i>mglA</i>	4.2E-06	-2.1
FTL_1025	FTT1061c	30S ribosomal protein S18	<i>rpsR</i>	1.2E-03	-2.1
FTL_0679	FTT0564	polyamine transporter, subunit I, ABC transporter, membrane protein	<i>potI</i>	1.0E-02	-2.2
FTL_0045	FTT1648c	Orotidine 5'-phosphate decarboxylase	<i>pyrF</i>	1.6E-07	-2.2
FTL_1118	FTT1085	hypothetical protein		1.9E-05	-2.2

FTL_1868	FTT1727c	multidrug resistance protein, membrane located		2.4E-02	-2.2
FTL_0685	FTT1259	NH(3)-dependent NAD(+) synthetase	<i>nadE</i>	1.5E-06	-2.3
FTL_1190	FTT1270c	Chaperone protein grpE (heat shock protein family 70 cofactor)	<i>grpE</i>	3.6E-03	-2.3
FTL_0337	NA <sup>b</sup>	pseudogene		2.0E-08	-2.4
FTL_0843	FTT1120c	queuine tRNA-ribosyltransferase.	<i>tgt</i>	1.4E-05	-2.4
FTL_0863	FTT0598c	Sodium-dicarboxylate symporter family protein		5.3E-07	-3.0
FTL_1478	FTT1317c	Inosine-5-monophosphate dehydrogenase	<i>guaB</i>	1.3E-05	-3.1
FTL_0382	FTT0881c	amino acid permease		1.9E-08	-3.5

<sup>a</sup>The open reading frame (ORF) designations are adopted from the annotations of the genomes of the LVS (GenBank Genome Accession No. NC\_007880) and Schu S4 strain (GenBank Genome Accession No. NC\_006570).

<sup>b</sup>NA denotes the absence of an ortholog in the indicated strain.