

1 **Mutation of *wbtJ*, a *N*-formyltransferase involved in O-antigen synthesis,**
2 **results in biofilm formation, phase variation, and attenuation in *Francisella***
3 ***tularensis*.**

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17 **SUPPLEMENTARY MATERIALS**

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20 **Materials and Methods**

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22 Table S1: Primers used in this study.

Primer Name	Sequence	Purpose
oKM189	cccGATCCATTAATGATGATAGTCTTG	<i>wbtJ</i> (FTL_0602) proximal F for deletion (bamHI)
oKM192	ggttGATATCGTAGTGTCGTTTGTATGATAC	<i>wbtJ</i> (FTL_0602) distal R for deletion (ecoRV)
oRGT_wbtJ_SOE1R	cccgggcccgggcccCATGAGGAAACCTCTTTGATATTATTA	deletion of <i>wbtJ</i> upstream flank use with oKM189
oRGT_wbtJ_SOE2F	gggcccggggcccgggTAGAAAAATGAGCCTTAAAAAATAC	deletion of <i>wbtJ</i> downstream flank use with oKM192
oKM193	cccGATCCCGATAAAATTAATAAAATAATT	<i>wbtJ</i> (FTL_0602) Forward complementation (ecoRV)
oRGT_wbtJ_seq F	gagccaaagcagtatatgccaa	Sanger sequencing of <i>wbtJ</i> region
oRGT_wbtJ_seq R	caatcagaccaaatgcatcatgac	Sanger sequencing of <i>wbtJ</i> region

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25 Table S2: Plasmids used in this study.

Plasmid Name	Description	Source
pEDL50	suicide vector for allelic exchange	Lovullo et al., 2012
pKM41	pEDL50 containing <i>wbtJ</i> deletion fragment	This study
pMP814	empty vector with <i>blaB</i> promoter	Lovullo et al., 2009
pKM43	pMP814 containing a functional copy of <i>wbtJ</i>	This study

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28 **Generation of growth curves.** *F. tularensis* strains were resuspended in PBS (phosphate buffered saline,
 29 pH 7.2) to an OD₆₀₀ of 0.3. Bacterial suspensions were diluted 1 to 10 into or Chamberlain's Defined
 30 Medium (CDM)¹ in a 96-well plate. Growth was then assayed by OD₆₀₀ reading every 30 min using a Tecan
 31 M200 Pro (Tecan Systems, San Jose, CA) microplate reader at 37°C with orbital shaking. Absorbance values
 32 were determined using the average of triplicate wells and subtracting the medium background as
 33 determined by the sterility control.

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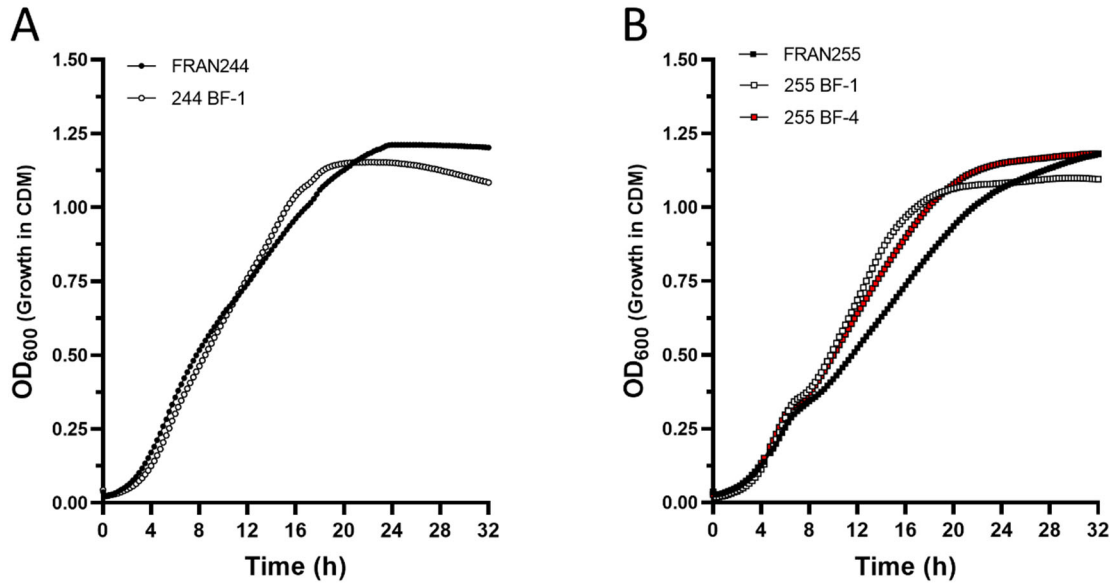
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40 **Results**

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44 **Figure S1: Growth curve analysis of biofilm forming variants compared to the parental isolate. (A)**

45 FRAN244 or **(B)** FRAN255 variant isolates were cultured in CDM at 37°C shaking over the course of 32 h

46 using the OD₆₀₀ to monitor growth. Data are the average of 2 independent experiments based upon 3

47 technical replicates per assay.

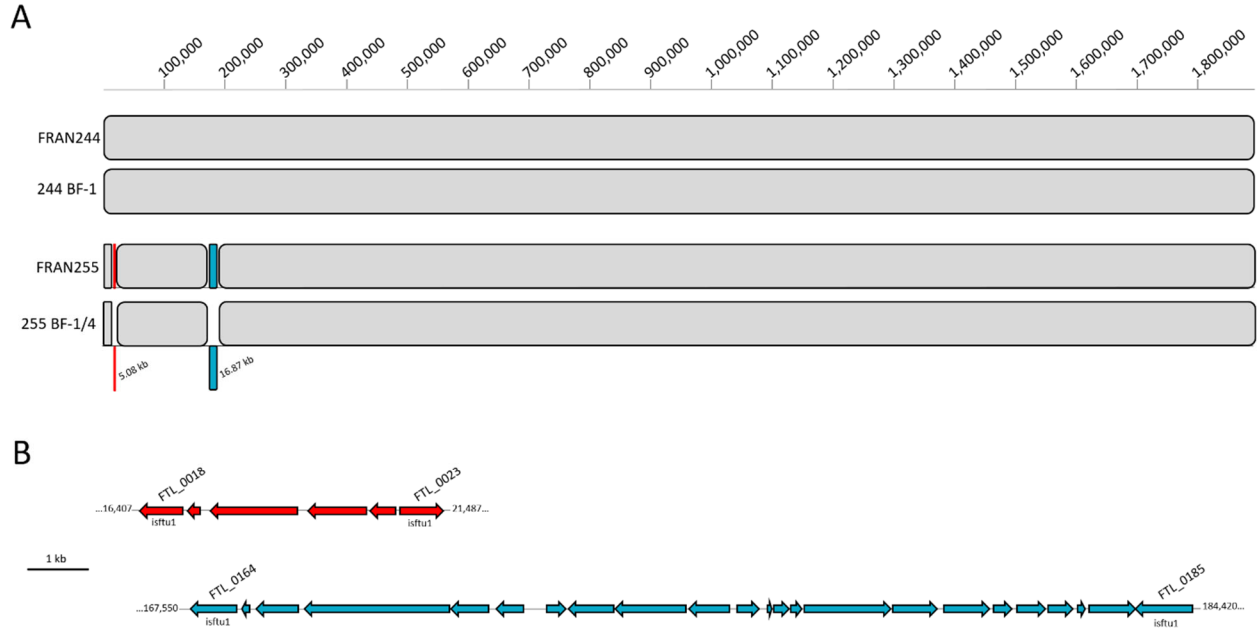
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Mutation of *wbtJ* in *F. tularensis*: Supplementary Materials



52 LVS genome co-ordinates displayed for ease of comparison (NC_007880)

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54 **Figure S2: Genome alignment of biofilm forming variants.** Illumina and Nanopore reads were used to
 55 generate *de novo* assemblies via Unicycler. Mauve was used for whole genome alignments compared to
 56 the respective parental strain. Two identical inversions were identified in 255 BF-1 and 255BF-4 bounded
 57 by repetitive mobile elements (*isftu1*) spanning 5080 bp (red invert) and 16, 870 bp (blue invert). For
 58 comparison purposes, LVS genome co-ordinates are shown.

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60 **Table S3:** SNPs identified in LVS gray variants that do not form biofilm as a control comparison.

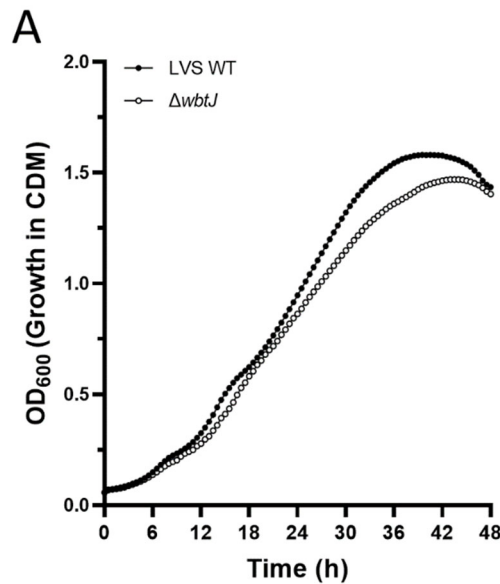
STRAIN	PHENOTYPE	POSITION	REF	ALT	STRAND	NT POS	AA POS	EFFECT	MUTATION	LOCUS TAG	KEGG	SchuS4 HOMOLOG	GENE	DESCRIPTION
LVS Isolate #15	GV, biofilm +	108388	C	T				intergenic		AW21_128	FTL_1339	FTT_0572		potential promotor alteration formyl transferase encodes 5sRNA
		1315079	C	T	-	457/726	153/241	nonsense	Gln153*	AW21_1477	FTL_0602	FTT_1454c	<i>wbtJ</i>	
		1848663	G	A		14/115		unknown		AW21_2092	FTL_R0032		<i>rfj</i>	
LVS Isolate #11	GV, biofilm -	1303079	G	T	-	292/1737	98/579	Nonsense	Glu98*	AW21_1467	FTL_0592	FTT_1464c	<i>wbtA1</i>	polysaccharide biosynthesis protein
LVS Isolate #13	GV, biofilm -	960093	T	G	+	458/624	153/208	missense	Val153Gly	AW21_1081	FTL_0237	FTT_0326	<i>rplD</i>	50s ribosomal protein L4
		1309532	T	G	-	194/1230	65/410	missense	Phe65Cys	AW21_1473	FTL_0598	FTT_1458c	<i>wzy</i>	Membrane protein/O-antigen protein
		1636790	C	A	+	423/633	141/211	synonymous	Ala	AW21_1838	FTL_0942	FTT_0668		nicotinamide mononucleotide transporter pmuC
LVS Isolate #14	GV, biofilm -	452704	G	T	-	348/474	116/158	synonymous	Gln	AW21_509	FTL_1693	FTT_1625c		putative pilus assembly protein
		1419600	T	C	+	449/1272	150/424	missense	Phe150Cys	AW21_1590	FTL_0705	FTT_1239		putative membrane protein
		1420302	C	T	-	47/1332	16/444	missense	Ala16Val	AW21_1591	FTL_0706	FTT_1238c	<i>waxL</i>	o-antigen ligase like membrane family protein

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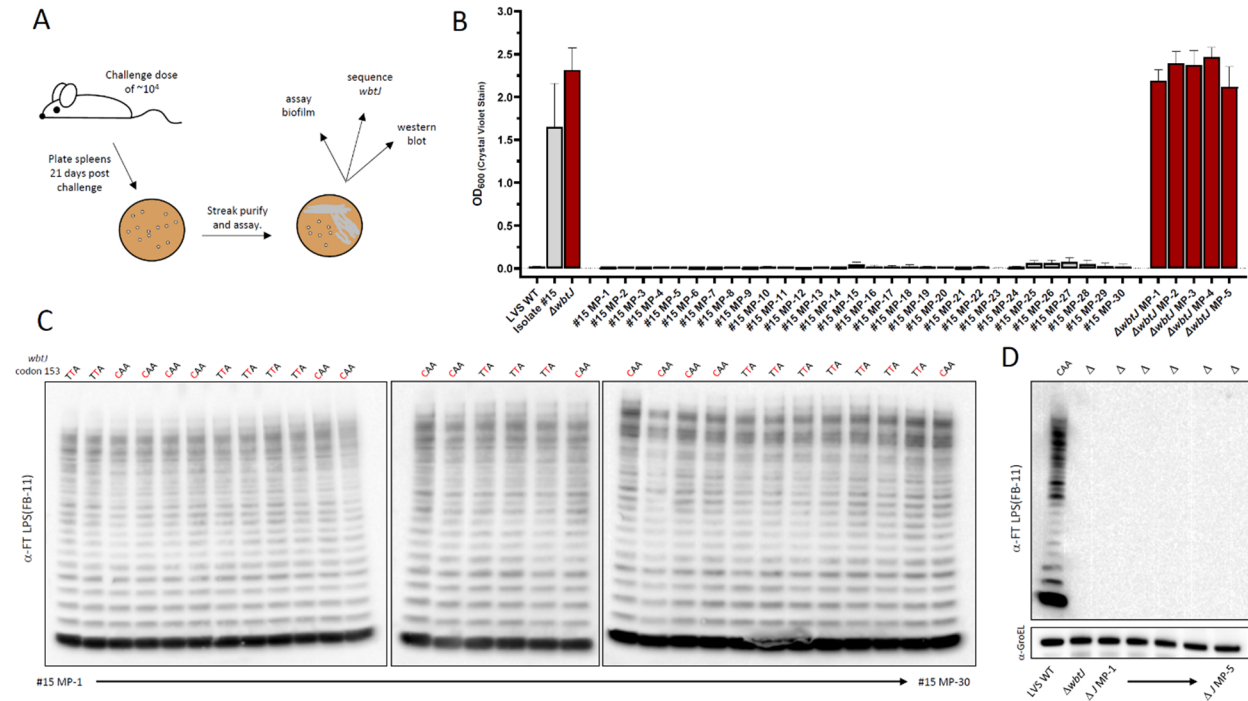
67 **Figure S3:** Growth curve analysis of an in-frame $\Delta wbtJ$ deletion mutant compared to the parental

68 isolate. LVS wild-type (parent, black circles) or an isogenic $\Delta wbtJ$ mutant (open circles) was cultured in

69 CDM at 37°C shaking over the course of 48 h using the OD₆₀₀ to monitor growth. Data are the average of

70 3 independent experiments based upon at least 3 technical replicates per assay.

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73 **Figure S4: Phenotypic assessment of colonies recovered from mouse spleen at 21 days post challenge**

74 **for Isolate #15 and a $\Delta wbtJ$ mutant. (A)** A schematic illustrating how samples were recovered and analyzed

75 at the end of study for Isolate #15 and a $\Delta wbtJ$ mutant to determine the *wbtJ* allele, biofilm forming

76 capacity, and O-Ag status. **(B)** Biofilm formation of purified representative isolates recovered from mice

77 spleens after challenge. Crystal violet staining was performed at two days post inoculation after static

78 incubation at 37°C. Western analysis with α -LPS (FB-11) on mouse passaged samples from **(C)** isolate #15

79 and **(D)** $\Delta wbtJ$ mutant. The sequence at the codon mutated in *wbtJ* is indicated above each lane. In the

80 case of $\Delta wbtJ$ isolates, α -GroEL was used as a loading control and *wbtJ* was confirmed to be absent by

81 sequencing. These data support the summary data provided in Table 6.

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84 **References**

85 1. Chamberlain, R.E. Evaluation of Live Tularemia Vaccine Prepared in a Chemically Defined
86 Medium. *Appl Microbiol* 13, 232-235 (1965).