1	Formation of the Francisella tularensis Biofilm is Affected by Cell Surface Glycosylation,
2	Growth Medium, and a Glucan Exopolysaccharide
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5	Anna E. Champion, Kelly C. Freudenberger Catanzaro, Aloka B. Bandara, and Thomas J. Inzana
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8	Supporting Information
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11 Supplementary Table S1. *Francisella novicida* transposon library mutant strains used.

Bacterial Strain	Locus Tag	Gene Name	Description
tnfn1_pw060328p01q189	FTN_1420	wzx	O antigen flippase
tnfn1_pw060323p06q119	FTN_1421	wbtH	Glutamine
			amidotransferase/asparagine
			synthase
tnfn1_pw060323p06q136	FTN_1422	wbtN	Glycosyl transferase, group 1
tnfn1_pw060323p03q189	FTN_1423	wbtG	Glycosyl transferase, group 1
tnfn1_pw060328p05q111	FTN_1424	wzy	Hypothetical membrane protein
tnfn1_pw060323p06q161	FTN_1425	wbtF	NAD-dependent epimerase
tnfn1_pw060328p03q164	FTN_1426	wbtE	USD-glucose/GDP-mannose
			dehydrogenase family protein
tnfn1_pw060419p04q192	FTN_1427	wbtD	Glycosyl transferase, group 1
tnfn1_pw060323p03q182	FTN_1428	wbtO	Transferase
tnfn1_pw060328p05q149	FTN_1429	wbyP	Galactosyl transferase
tnfn1_pw060419p04q158	FTN_1430	wbtQ	Aminotransferase
tnfn1_pw060323p06q123	FTN_1431	wbtA	dTDP-glucose 4,6-dehydratase

16	Supplementary Fig. S1. Glycose composition and linkage analysis of the EPS from F.
17	tularensis by gas-chromatography-mass spectrometry (GC-MS). The purified EPS was
18	permethylated, hydrolyzed, reduced, and acetylated as described in Methods. The methylated
19	alditol acetates were separated on a 30 m Supelco SP-2331 bonded phase fused silica capillary
20	column, and analyzed on an Agilent 7890A GC interfaced to a 5975C mass selective detector.
21	Glycose analysis by GC-MS (top panel) showed that 2 monosaccharides were identified,
22	consisting of 99.9% glucose and 0.1% mannose. Linkage analysis (bottom panel) indicated that
23	54% of the glucose residues were terminal, 33% were 1,4-linked, and 1.6% were 1,6-linked,
24	which is consistent with amylose. A minor component (4.4%) of the EPS was 1,4-linked
25	mannose.
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27	Supplementary Fig. S2. Static biofilm development by <i>F. tularensis</i> Type A strains in A:
27 28	Supplementary Fig. S2. Static biofilm development by <i>F. tularensis</i> Type A strains in A: MHB or B: CDMB. Differences in biofilm development between Type A strains SCHUS4 and
27 28 29	Supplementary Fig. S2. Static biofilm development by <i>F. tularensis</i> Type A strains in A:MHB or B: CDMB. Differences in biofilm development between Type A strains SCHUS4 andclinical isolate TI0902 were not significant whether grown in MH medium (A) or CDM medium
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27 28 29 30 31 32	Supplementary Fig. S2. Static biofilm development by <i>F. tularensis</i> Type A strains in A:MHB or B: CDMB. Differences in biofilm development between Type A strains SCHUS4 andclinical isolate TI0902 were not significant whether grown in MH medium (A) or CDM medium(B) $(p > 0.5)$. However, O-Ag mutant TIGB03 made significantly more biofilm after 5 and 10days incubation in MH medium $(p < 0.01)$, and after 5 days incubation in CDM medium (surface $p < 0.05$). However, the amount of biofilm made by SCHUS4 and TI0902 increased enough by
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27 28 29 30 31 32 33 34 35	Supplementary Fig. S2. Static biofilm development by <i>F. tularensis</i> Type A strains in A: MHB or B: CDMB. Differences in biofilm development between Type A strains SCHUS4 and clinical isolate TI0902 were not significant whether grown in MH medium (A) or CDM medium (B) ($p > 0.5$). However, O-Ag mutant TIGB03 made significantly more biofilm after 5 and 10 days incubation in MH medium ($p < 0.01$), and after 5 days incubation in CDM medium (surface p < 0.05). However, the amount of biofilm made by SCHUS4 and TI0902 increased enough by day 10 that there was not a significant difference in biofilm formation. Significance was determined by unpaired <i>t</i> test.
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38 Supplementary Fig. S3. Surface and broth extractions of LVS strains grown as biofilms.

- 39 Each bacterial strain/mutant was grown in CDMB for 25 days, the medium above the biofilm
- 40 was removed, the planktonic cells harvested by centrifugation, proteins in the medium
- 41 precipitated by addition of 3 volumes of 95% ethanol, and the precipitate resuspended in distilled
- 42 water. Planktonic cells in the medium and cells in the biofilm were extracted with 1M urea. The
- 43 protein profiles were resolved by SDS-PAGE on 4-12% gels, stained with silver. L: LVS, P:
- 44 LVS P10, LM: LVSΔ1423-22, W: WbtIG191V, WM: WbtIG191VΔ1423-22, M: Ladder.
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47 Supplementary Fig. S1.





50 Supplementary Fig. S2.

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53 Supplementary Fig. S3.