Supplemental Results.

ChiA: Homologues of *F. novicida chiA* were identified in *F. tularensis* A1a, A1b, A2 and type B. The *F. tularensis* A1a/A1b and type B, and *F. novicida chiA* genes were predicted to encode proteins of 760, 760 and 870 aa, respectively (Fig. 2A). The *F. tularensis* A2 *chiA* gene is annotated as a pseudogene and is predicted to encode a truncated product of 198 aa (2). All the predicted intact ChiA proteins contained a full length GH18 domain (PfamID: PF00704). Three accessory domains, fibronectin type 3 domain (Pfam ID: PF00041) and two tandem carbohydrate binding domains (PfamID: PF02839), were identified in the ChiA sequences of *F. tularensis* A1a/A1b and type B, and *F. novicida*. The *F. novicida* ChiA, however, possessed a third carbohydrate binding domain that partially accounts for its larger size. All accessory domains are located C-terminal to the GH18 domain. All *chiA* gene products were predicted to possess a N-terminal signal peptide suggesting they are translocated across the cytoplasmic membrane.

ChiB: The genomes of *F. tularensis* A1a, A1b and type B, and *F. novicida* possessed the *chiB* gene encoding chitinases of 606, 730 and 730 aa, respectively, and that contained an intact GH18 domain. *F. tularensis* A2 displayed an altered *chiB* gene resulting in a product with a 566 aa truncation that accounts for the absence of the GH18 domain (Fig. 2B). A partial *N*-acetylglucosamine-binding protein A domain (conserved domain (cdd) ID: PRK13211) was identified at the N-terminus of all predicted ChiB proteins except that of the *F. tularensis* A1a/ A1b where a 124 aa N-terminal truncation occurs. This truncation also encompassed the signal peptide conserved in the other ChiB sequences of *F. tularensis* A2 and type B, and *F. novicida*.

ChiC: The third chitinase (ChiC) identified in *F. tularensis* A1a/1b, A2, type B, and *F. novicida* differed significantly when compared across the subtypes. The *F. tularensis* A2 and *F. novicida* strain GA99-3548 *chiC* genes encode a predicted full length products of 762 aa. The *F. tularensis* type B ChiC contained a C-terminal truncation of 58 aa that impacts the GH18 domain. Most interestingly, the *F. tularensis* A1a/A1b *chiC* gene possessed a point mutation that causing a premature stop codon and two predicted open reading frames. In *F. tularensis* A1a these two reading frames were annotated as FTT_1592c and FTT_1593c and encode products of 387 and 207 aa, respectively. The *F. tularensis* A1a FTT_1592c open reading frame encodes for the C-terminal portion of ChiC that includes a complete GH18 domain (Fig. 2C). The ChiC proteins also possess two tandem carbohydrate binding domains (PfamID: PF02839). The position of these domains is directly after the N-terminal signal sequence, a location that is in sharp contrast to those of the ChiA proteins.

ChiD: ChiD was highly conserved among all *Francisella* spp. and subtypes (Fig. 2D). All were predicted to contain a full length C-terminal GH18 domain, and an incomplete second GH18 domain. A partial *N*-acetylglucosamine-binding protein A domain was also predicted at the C-terminus. It should be noted that the shotgun genome sequence used by others to assemble the *F. tularensis* A1b genome found several alterations in *chiD*, including a mutation leading to a premature stop codon. However, our conventional sequencing of *chiD* amplified by PCR from *F. tularensis* A1b strain MA00-2987 found it to be identical to the *F. tularensis* A1a *chiD*.

TABLE S1. Primers used to generate histidine tagged chitinase recombinants

Chitinase class	Loci[a]	Amplicon size	Forward primer (5′-3′)[b]	Reverse Primer (5′-3′)[b]
ChiA	FTT_0715	2292	GCT AGC ATG AAC AAA ACA AAA TTA GTC TCA GTA G	GTC GAC TTG TTT TTC CCA AAC ATT AC
	FTH_1471	2292	GCT AGC ATG AAC AAA ACA AAA TTA GTC TCA GTA G	GTC GAC TTG TTT TTC CCA AAC ATT AC
	FTN_0627	2622	GCT AGC ATG AAC AAA ACA AAA TTA	GTC GAC TTG TTT TTC CCA AAC
ChiB	FTT_1768c	1830	GCT AGC ATG CCA TAT TCT GAT ACC C	CTC GAG TTT ATC ATT TAT AGG ATA A
	FTH_0088	2202	GCT AGC ATG AAA TAC AAA AAG TTA TTA	CTC GAG TTT ATC ATT TAT AGG ATA A
	FTN_1744	2202	GCT AGC ATG AAA TAC AAA AAG TTA TT	CTC GAG TTT ATC ATT TAT AGG ATA AA
ChiC	FTT_1592c	1173	ACT AGT GTG ACT GGA TAT AAA GCT ATC	CTC GAG TTT AGA AGT ATA CTT TTC TTG AG
	FTW_0313	2298	ACT AGT ATG AAA AAA ATG AAA TTA ATC TCA TC	CTC GAG TTT AGA AGT ATA CTT TTC TTG AG
	FTH_1579	2049	ACT AGT ACT ACT ATT AAA TCA GCA TCA TC	CTC GAG TCG ACT TCC ATG CC
ChiD	FTT_0066	2853	GCT AGC ATG AGA AAA CTT TTT ATA A	CTC GAG TTT ACT ATC TAT TTT TGT CCA
	FTT_0142	2853	GCT AGC ATG AGA AAA CTT TTT ATA A	CTC GAG TTT ACT ATC TAT TTT TGT CCA
	FTT_1730	2853	GCT AGC ATG AGA AAA CTT TTT ATA A	CTC GAG TTT ACT ATC TAT TTT TGT CCA
	FTN_1644	2853	GCT AGC ATG AGA AAA CTT TTT ATA A	CTC GAG TTT ACT ATC TAT TTT TGT CCA

[a] FTT corresponds to A1a/A1b strains; FTW corresponds to A2 strains; FTH corresponds to type B strains; FTN corresponds to *F. novicida* strains.
[b] The *NheI* (GCT AGC), *SpeI* (ACT AGT), *SalI* (GTC GAC) and *XhoI* (CTC GAG) restriction sites are underlined.

TABLE S2. Plasmids used in this study

Plasmid[a]	Description	Source
pCR®-Blunt II-TOPO®	Km^R, Zeo^R, cloning vector	Invitrogen
pET23b-(+)	Ap^R, Histidine tagged, expression vector	Novagen
pET23b-(+)_FTT_0715	A1a/A1b *chiA* expression vector	This study
pET23b-(+)_FTH_1471	type B *chiA* expression vector	This study
pET23b-(+)_FTN_0627	*F. novicida chiA* expression vector	This study
pET23b-(+)_FTT_1768c	A1a/A1b *chiB* expression vector	This study
pET23b-(+)_FTH_0088	type B *chiB* expression vector	This study
pET23b-(+)_FTN_1744	*F. novicida chiB* expression vector	This study
pET23b-(+)_FTT_1592c	A1a/A1b *chiC* (GH18 fragment) expression vector	This study
pET23b-(+)_FTW_0313	A2 *chiC* expression vector	This study
pET23b-(+)_FTH_1579[b]	type B *chiC* (no signal peptide) expression vector	This study
pET23b-(+)_FTT_0066	A1a/A1b *chiD* expression vector	This study
pET23b-(+)_FTW_0142	A2 *chiD* expression vector	This study
pET23b-(+)_FTH_1730	type B *chiD* expression vector	This study
pET23b-(+)_FTN_1644	*F. novicida chiD* expression vector	This study
pMP590	*sacB*-based allelic exchange vector	LoVullo(25)
pMP529	*Francisella* shuttle/integration vector	LoVullo(25)
pMP590-Δ*chiA*	allelic exchange vector for *chiA* knockout	This study
pMP590-Δ*chiC*	allelic exchange vector for *chiC* knockout	This study
pMP529-*chiA*	*chiA* integration vector	This study
pMP529-*chiC*	*chiC* integration vector	This study

[a] FTT, FTW, FTH and FTN loci correspond to those listed in Supplementary Table 1.
[b] Attempts to produce protein with the signal peptide failed.

TABLE S3. Internal sequencing primers (5′-3′)

Loci	Primer 1	Primer 2	Primer 3	Primer 4
FTT_0715	GTA TGG AAA ACT TTG CTA AGC AGT	GTT GCA TTG CTA CTT TAC CGT ACT TGT TAA	-	-
FTH_1471	GTA TGG AAA ACT TTG CTA AGC AGT	GTT GCA TTG CTA CTT TAC CGT ACT TGT TAA	-	-
FTN_0627	GTA TGG AAA ACT TTG CTA AGC AGT	GTT GCA TTG CTA CTT TAC CGT ACT TGT TAA	CGC TAG ATC AAT GAC TGT AGC TAG TG	-
FTT_1768c	GAG CCT ATA ACT TTA GGC AAG GG	GAT AAA GTT GCA GGA CCA CTT CT	TTT ACG AAA CAC GGA AAC CGA A	-
FTH_0088	GAG CCT ATA ACT TTA GGC AAG GG	GGG TGA GTT TAC AAT TAA GCC AG	-	-
FTN_1744	GGG TGA GTT TAC AAT TAA GCC AG	ACA ACT GGC TTA CCT CAA ACT AT	-	-
FTT_1592c	-	-	-	-
FTT_1593c	-	-	-	-
FTW_0313	GTT ATT GCT GAA GTG AAA GAT GCT A	AGC AAC AGT CTC GCC AGC TGA G	GAC GTT AGA TGG TTC ACC AAA TCC T	CTG CAG AAG ATG TGG CTC CTT AC
FTH_1579	GTT ATT GCT GAA GTG AAA GAT GCT A	GAC GTT AGA TGG TTC ACC AAA TCC T	-	-
FTT_0066	TAA TTG CTG CTG AAC CAG AAG T	CAT GGG CCA GTT GTT GCT GG	GTA GTT CTG ATA TGC CTA AGA ATG AT	GGA TAC ATT CCA AAT GGA CTA TAT GG
FTW_0142	TAA TTG CTG CTG AAC CAG AAG T	CAT GGG CCA GTT GTT GCT GG	GTA GTT CTG ATA TGC CTA AGA ATG AT	GGA TAC ATT CCA AAT GGA CTA TAT GG
FTH_1730	TAA TTG CTG CTG AAC CAG AAG T	CAT GGG CCA GTT GTT GCT GG	GTA GTT CTG ATA TGC CTA AGA ATG AT	GGA TAC ATT CCA AAT GGA CTA TAT GG
FTN_1644	TAA TTG CTG CTG AAC CAG AAG T	CAT GGG CCA GTT GTT GCT GG	GTA GTT CTG ATA TGC CTA AGA ATG AT	GGA TAC ATT CCA AAT GGA CTA TAT GG

TABLE S4. Primers used for constructing knockouts and complemented knockouts

Construct	Fragment amplified	Amplicon size (bp)	Forward primer (5′-3′)[b]	Reverse Primer (5′-3′)[b]
pMP590-Δ*chiA*	*chiA* upstream	562	TTT GAC TAA GGA TCC ATA TTA AC	TCT AGC AGT CGA CAT GAT CC
	chiA downstream	941	AAG TAG CTG CAG ACC AAG GC	AGT TAG GAT CCA ATT ACT AAT AG
pMP590-Δ*chiC*[a]	*chiC* upstream	477	ATG AGT ACG CGT ATG ATG CTG	ATC CTC GTC GAC AAC ACG G
	chiC downstream	608	TAA CAC AGT CGA CAT TTA TGG	AGG AAA CGC GTC ATA CTA TC
pMP529-*chiA*	*chiA*	2918	AAT ACG CGT TCA TGT GAG CTC CTT TAA GC	TAT ACG CGT TAT CTT CAT AAA TTA TGC GC
pMP529-*chiC*[a]	*chiC*	2298	ACG CGT ATG AAA AAA ATG AAA TTA ATC TCA TC	ACG CGT TTT AGA AGT ATA CTT TTC TTG AG

[a]Primers designed using complement sequence.

[b]The *BamHI* (GGA TCC), *SalI* (GTC GAC), *PstI* (CTG CAG) and *MluI* (ACG CGT) restriction sites are underlined.