

Table S1: Bacterial strains and plasmids

Strain/plasmid	Genotype/Description	Reference
<i>F. novicida</i> strain		
U112	wildtype	(1)
KKF457	Δ (FTN0451-FTN0456):: <i>ermC</i>	This study
KKF535	<i>cdgB</i> ::ISFn2/FRT	This study
KKF538	<i>cdgA</i> ::ISFn2	This study
KKF539	<i>cdgA</i> ::ISFn2; <i>cdgB</i> ::FRT	This study
KKF552	<i>qseB</i> :: ISFn2/FRT	This study
KKF555	<i>chiA</i> :: ISFn2/FRT	This study
KKF556	<i>chiB</i> :: ISFn2/FRT	This study
KKF557	Δ (FTN0451-FTN0456):: <i>ermC</i> ; <i>chiA</i> :: ISFn2/FRT	This study
KKF558	Δ (FTN0451-FTN0456):: <i>ermC</i> ; <i>chiB</i> :: ISFn2/FRT	This study
Plasmids		
pKEK894	<i>Francisella</i> expression vector; Tet ^R	(2)
pKEK1112	Expresses FLP recombinase; Tet ^R ; ts	(2)
pKEK1524	<i>cdgB</i> in pKEK894	This study
pKEK1525	VCA0956 in pKEK894	This study

Table S2: Primers used in this study

Primer	Sequence 5'-3' (restriction site/universal priming site underlined)
Δ(451-456) 1	GCTATCCTATTGAGCAAATTACTACAGTGG
Δ(451-456) 2	<u>CTCAACGGCCTCAACCTACTACTGGGCTTCTATAGGCTGTAATTTT</u> TATCAAG
Δ(451-456) 3	<u>ACTGCAGGGATCCGGCTGCTAACAAAGCGAGCAATAATATCGCTT</u> GCAGAGAGTTTA
Δ(451-456) 4	GTAAGAGTCTCAATAAACTCAGCAAGCGTTGAGC
cdgBNcoI:	<u>CGCGCGCCATGGGCGTGATAAAGAAGTCTTATGATTGG</u>
cdgBEcoRI:	<u>CGGAATTCTTAACTAGAAGTATGATTTTAAATTT</u>
VCA0956NcoI:	<u>CGCGCGCCATGGGCGTGATGACAAGTGAAGATTTCAAAAAATC</u>
VCA0956EcoRI:	<u>CGGAATTCTTAGAGCGGCATGACTCGATTGCGGCC</u>
cdgA rtF	AAGCACCTAGCTGACCCAAGTTAT
cdgA rtR	CCACACGATCGAGCCATTTCTATG
cdgB rtF	CATCACGCAAGCATGATAAATATTT
cdgB rtR	CTTCCCAAGCTCCTTGTGATTTAAG
chiA rtF	GCCAGGTGTGGTTGTTCTATC
chiA rtR	CATAGTATGCAGCATTAGTGTATC
chiB rtF	ACTTTGCCATATTCTGATACCCAAG
chiB rtR	TCACACTTTCCAGGTAAGTGGTCCG
FTN0450 rtF	CTAATGCTGATATCCATACCAGTCAAAC
FTN0450 rtR	TAGCACTGGCAAAGCTTCTTTGAGCGTC
rpoB rtF	AGGGCATAACTTGATGGTTGCT
rpoB rtR	CGAGCCACACATGTGAACTCT

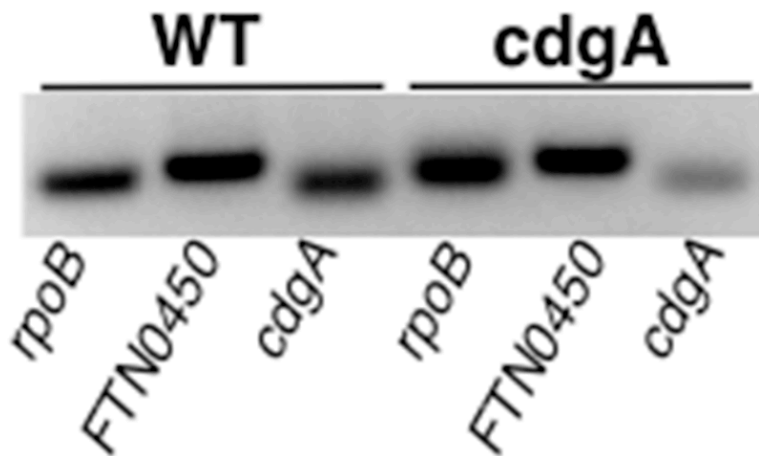


Figure S1: Transposon insertion in *cdgA* does not adversely affect FTN0450 transcription. *F. novicida* strains U112 (WT) and KKF538 (*cdgA::ISFn2*) were grown in CDM, and mRNA abundance for *FTN0450* and *cdgA*, as well as *rpoB* (control) was determined by reverse transcription-PCR (see Materials & Methods). Results indicate similar levels of *FTN0450* transcript in both the wildtype and *cdgA* mutant strains. In contrast, the Tn insertion in *cdgA* (which lies at nucleotide 352 within the *cdgA* gene) leads to reduced *cdgA* transcript levels in KKF538 (nucleotides 256-330 of *cdgA* transcript amplified).

Fig. S2. Alignment of CdgA and CdgB with other DGCs (GGDEF domain-containing proteins)

CdgA	YKDDL T GLLNRKGF I KKASNHIA EIQN -LNLVTLFCIDLDNFKFINDSFG Y NIGDELI I K	340
CdgB	YQDMLTGLFNRY F ESHISDHIEK GARQ RQPFTLMFLDLDRFKEVNDVYGHQAGDKLLIS	405
VCA0956	LFDSL S GLYNRRAFD GDM FTLIH---AGQ Q VSLIMLDIDHFKALNDNYGHLFGDQ I IRA	236
PleD	-TDQ L TGLHNRRYMT GQ LD S LVKRATLGGDPVSALLIDIDFFKKINDTFGHDIGDEV L RE	349
WspR	--DGLTGLSNRRHFDEYLEMEWRR SLRE Q S QLSLLMIDVDYFKSYNDTFGHVAGDEAL RQ	231
	* * : * * * * : Is . . : : * : * * * * * : * : * : *	
CdgA	ISKRLKN-FF N KD A IIGRS GGDDF LILTDNLQSLIEIAQIA EN LIKEIAKPYIMKGN---	396
CdgB	VSNRLKE-LVREKDLVARL GGDEF LLFFVDMT-IDNAIKKAHKVVEYIAKPYDIDDK---	460
VCA0956	IAKRLQS-LCRDGV TAYRYGGEEF ALIAPHKS-LRIARQFAESVRRSIEKLT VKDRR SGQ	294
PleD	FALRLAS-NVRAIDLPCRY GGEEF VVIMPDTA-LADALRIAERIRMHVSGSPFTVAH-GR	406
WspR	VAGAIREGCSRSSDLAARY GGEEF AMVLPGTS-PGGARLLA E KVRR T VESLQISHDQ-PR	289
	. : : . . Ip * * * : * * : . * . : : * : .	
	Ip Is	
CdgA	-TFSQSSSIGIAI--YPNVADNFEKLIQFADTAMHHAKAKGKNTY-	438
CdgB	-QFIISASIGVVN--YPODGTDFEHLKYADAAMYRAKDLGRNRF-	502
VCA0956	SVGSITASFGVV---EKIEGDSLES L IGRADGLLYEAKNLGRNRV-	336
PleD	EMLNVTISIGVS--ATAGEGDTPEALLKRADEGVYQAKASGRNAVV	450
WspR	PGSHLTVSIGVSTLVPGGGGQ T FRVLIEMADQALYQAKNNGRNQV-	334
	: * : * : . . * : * * : : * * * : *	

Figure S2. Alignment of CdgA and CdgB with other DGCs (GGDEF-containing proteins).

ClustalW alignment of CdgA and CdgB with *V. cholerae* VCA0956 (Accession AAF96852), *C.*

cresecentus PleD (accession AAA87378) and *P. aeruginosa* WspR (accession NP_252391). Residues

with identity between all proteins are designated with asterisk, while residues with high similarity are

designated with a colon. The GGDEF motif, primary (Ip) and secondary (Is) inhibitory sites are noted.

Substitution of Y for highly conserved H in CdgA is shown in red, substitutions from consensus at Is

and Ip sites in CdgA are shown in blue.

Fig. S3. Alignment of CdgA and CdgB with other PDEs (EAL domain-containing proteins)

CdgA	OKTQRRNSIDRELRNAEMAEFTIVYQPQFDINN-EIYGV EAL VRWQSPKLGNISPEEFMP	505
CdgB	-----LNDAFENTDY--IQVLFQPOYDLKQRQIYGI EIL SRINHPDLGVVCPKDFIP	572
VieA	-----EIEQAFLHDHI---FNYYQPQFDFRSGAMVGV EAL VRYEHPHTGMLSPAFLP	190
BlrP1	-----QFALQAIVEPAKKRVSS F EAL IRSP---TGGSPVEMFAA	205
YkuI	-----DDVLPYYQAI F SAEEQKVVG Y EVL GRILADSEIQSLGPFLLD	53
	* . . . : . * * *	*
CdgA	IAEKNQTIKNIGKWIFIRTIQDWNKLLSLNLANNLKLSINVSSVQILOENFCS-EVINII	564
CdgB	IL Q SINQMPSPDKIVLQKACQOISSND-MMLEQNFRISQNVTVVPVIMSEQYCR-EFIDII	630
VieA	LIEQCGLHEKLF ^o TLVLEKSVSALASIG-----ADLQLSVNISQ-RNLQHSICD-PILAIC	243
BlrP1	IAAEDRYRFDLESKAYAFALAGQLPLG-----KHQLAINLLPGSLYHHPDAVGWLMDSL	259
YkuI	AGIPEEYKLEVDNRIIRQALDRFLEAD----SDLLIFXNQDANLLXLDHGESFLELLKEY	109
	. . : : . . . :	
CdgA	SN--IDKHSITL ^o EITETHLLANIDLTRTVISNMNKLGISFALDDFGTGYSSSLKYLANLPI	622
CdgB	KQYKISPQRFSFEVIENIAIDDYKTACKNFCLLKKEGISVEIDDFG S GYSSLSYLAQFPI	690
VieA	ERYGFPASKLTLEMTEHEVYNGTPTSLANLARLRMYGVGLSIDDFTGYASLGQLAQLPF	303
BlrP1	LAAGLRPDQVLI ^o EVTEVITCFDQFRKVLKALRVAGMKLAIDDFGAGYSGLSLLTRFQP	319
YkuI	EAKGIELHRFVLEITEHNFEGDIEQLYHXLAYRRTYGIKIAVDNIGKESNLDRIALLS	169
	: . : * : . * : . : * : * : * : *	: * : :
	Loop 6	
CdgA	DYLKIDKEFVQNLNIQ--NNKEIIIAITQLAKNLNKYCIAEGIETLEQFNFLKSIGCNFYQ	681
CdgB	TVLKIDSFFVQNIHHP-REEKICRAIISLAESLNLK ^o VIAEGVETKHQADTLYKMGCYLHQ	749
VieA	TELKIDRSFVHDLATNYKHQQLTNMCLLLAQSLGLHCVVEGVENEETWOYLRQLGVDTQC	363
BlrP1	DKIKVDAELVRDIHISGTKQAIVASVVRCCEDLGITVVAEGVETLEEW ^o CWLOSVGIRLFQ	379
YkuI	DLLKIDLQALKVSPSPSYEHVLYSISLLARKIGAALLYEDIEANFQLOYAWRNGGRYFQ	229
	* : * : : : . . . : * : * : *	* *
CdgA	GYFYSKPIF--	691
CdgB	GFLYSQPLSYEE	761
VieA	GYAAKPMPIAQ	375
BlrP1	GFLFSRP-----	386
YkuI	GYLVSP-----	236
	* : *	

Figure S3. Alignment of CdgA and CdgB with other PDEs (EAL domain-containing proteins).

ClustalW alignment of CdgA and CdgB with *V. cholerae* VieA (Accession NP_231289), *Klebsiella pneumoniae* BlrP1 (accession ABR77029) and *Bacillus subtilis* YkuI (CAA10872). Residues with identity between all proteins are designated with asterisk, while residues with high similarity are designated with a colon. The EAL motif and loop 6 are noted. Substitution of S in loop 6 DDFGTG motif, and Q for E in CdgA are shown in red.

Fig. S4. Alignment of FTN0452 with CdgA, CdgB, and VCA0956

```

FTN0452    TLTDKAKHYVRMNLLEYAMSIFDDINYIAPEFFYSIDWAYSMDQNRDKAYEFSLVQISIF 373
CdgA       YKDDL TGLLN RKGFIKKASNHIAEIQN-LNLVTLFCIDLNDN-----FKFINDSFG 330
CdgB       YQDML TGLFN RRYFESHISDHIEK GARQRQPFTLMFLDLDR-----FKEVNDVYG 395
VCA0956    LFDSL SGLYN RRAF DGMFTLIHAG----QQVSLIMLDIDH-----FKALNDNYG 226
           :   *   :       :           .   :*                   *.  ::

FTN0452    DYLSMADIRVLSKALRDVLRKTDMLTRISRHDIWLWLPNTQ-----LQGANIVIKKQI 428
CdgA       YNIGDELI I KISKRLKNFFNKDAI IGRSGGDDFLILTDNLQSLIEIAQIAENLIKEIAKP 390
CdgB       HQAGDKLLISVSNRLKELVREKDLVARLGGDEFLLFFVDMT-IDNAIKKAHKVVEYIAKP 454
VCA0956    HLFGDQI I RAI AKRLQSLCRDGV TAYRYGGEEFALIAPHKS-LRIARQFAESVRRSIEKL 285
           .   :   ::: *:. . . .   * . . :: :   .       : * . : . : :

FTN0452    NTTESS-----SIESAVKMTAYHSKALDDFTTAQKFLKKISY----- 465
CdgA       YIMKGN----TFSQSSSIGIAIYPNVADNFEKLIQFADTAMHHAKAKGKNTY 438
CdgB       YDIDDK--QFI--ISASIGVVNYPQDGTDFEHL LKYADAAMYRAKDLGRNRF 502
VCA0956    TVKDRRSGQSVGSITASFGVVEKIE-GDSLES LI GRADGLLYEAKNLGRNRV 336
           .           ::   .   :   ::   .   :

```

Figure S4. Alignment of FTN0456 with other DGCs. ClustalW alignment of FTN0456 with CdgA, CdgB, and *V. cholerae* VCA0956 (Accession AAF96852). Residues with identity between all proteins are designated with asterisk, while residues with high similarity are designated with a colon. The GGDEF motif is highlighted.

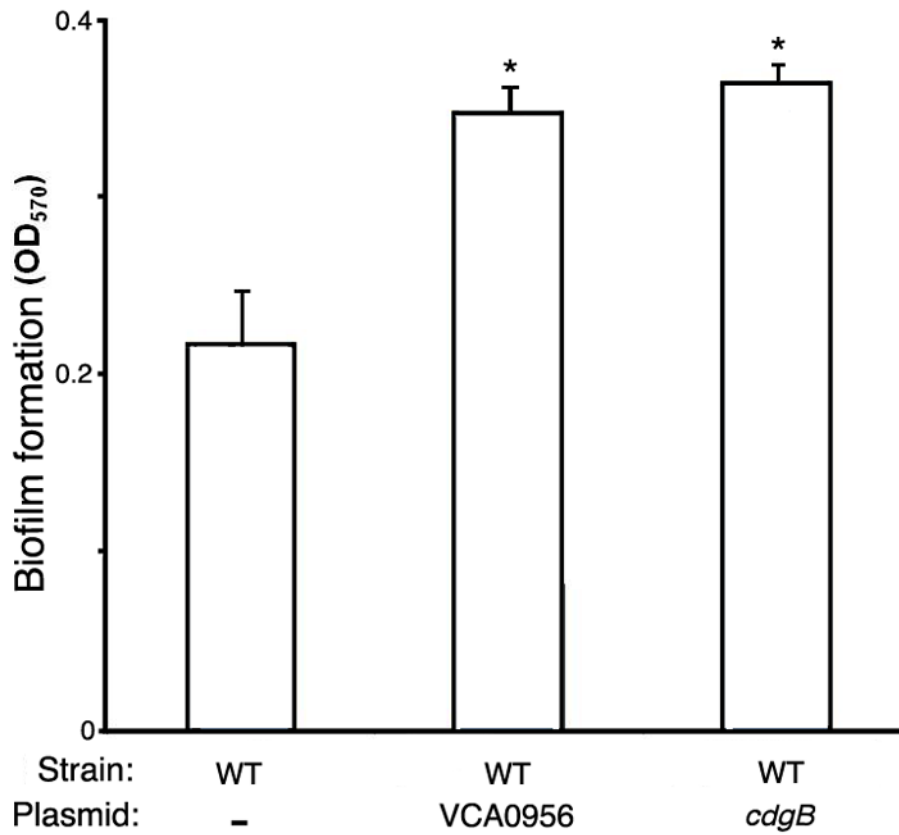


Figure S5: CdgB stimulates *V. cholerae* Biofilm formation. *V. cholerae* strain A1552 either with no plasmid (“-“) or carrying plasmids pKEK1524 (+*cdgB*) or pKEK1525 (+VCA0956) was assayed for biofilm formation as described in materials & methods. * $p < 0.05$

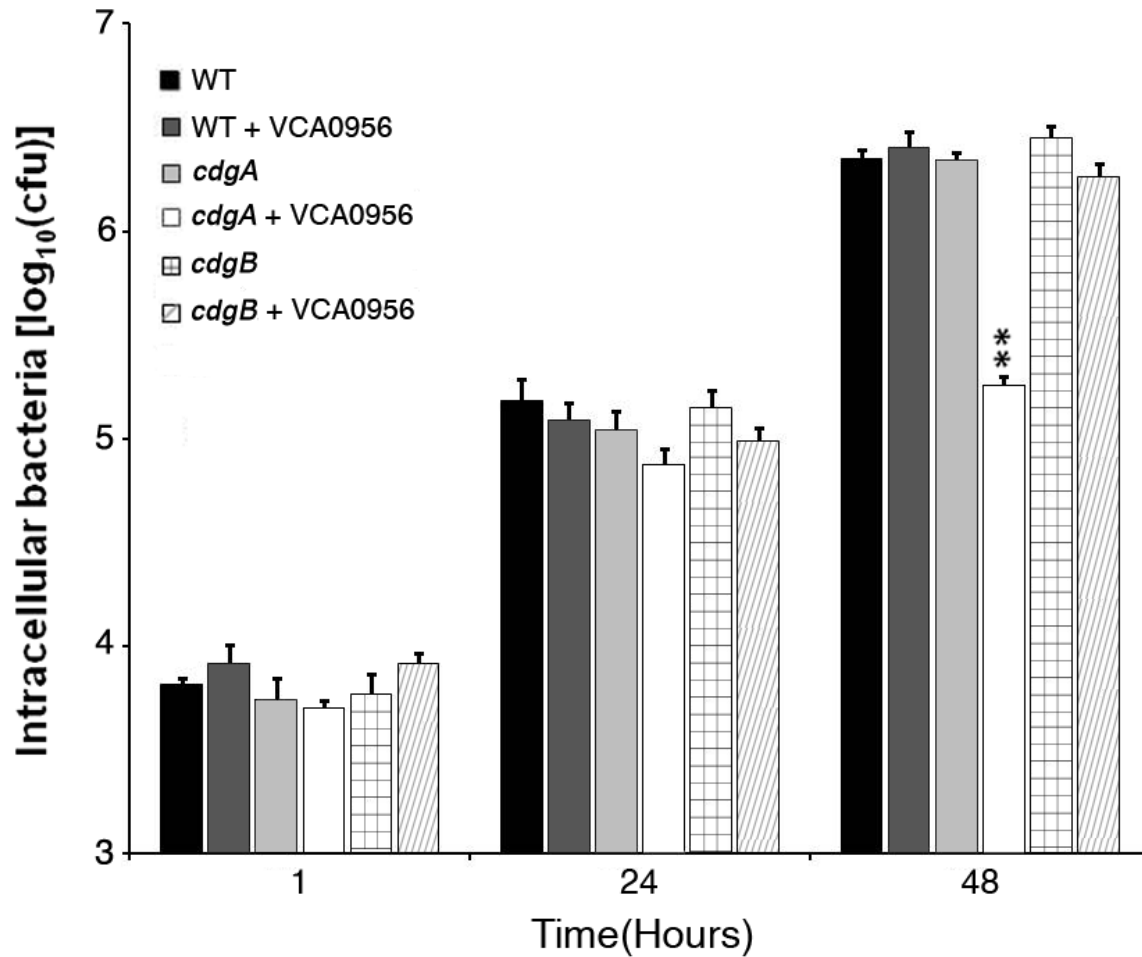


Figure S6. Lack of CdgA is required for cdGMP-mediated inhibition of *F. novicida* intramacrophage replication. *F. novicida* strains U112 (WT), KKF538 (*cdgA*), and KKF535 (*cdgB*) either without plasmid or carrying plasmid pKEK1525 (+VCA0956) were inoculated at an MOI of ~10:1 into J774 cells, and intracellular bacteria were enumerated at 1, 24, and 48 h. (see materials & methods). The assay was performed in triplicate. ** p <0.005.

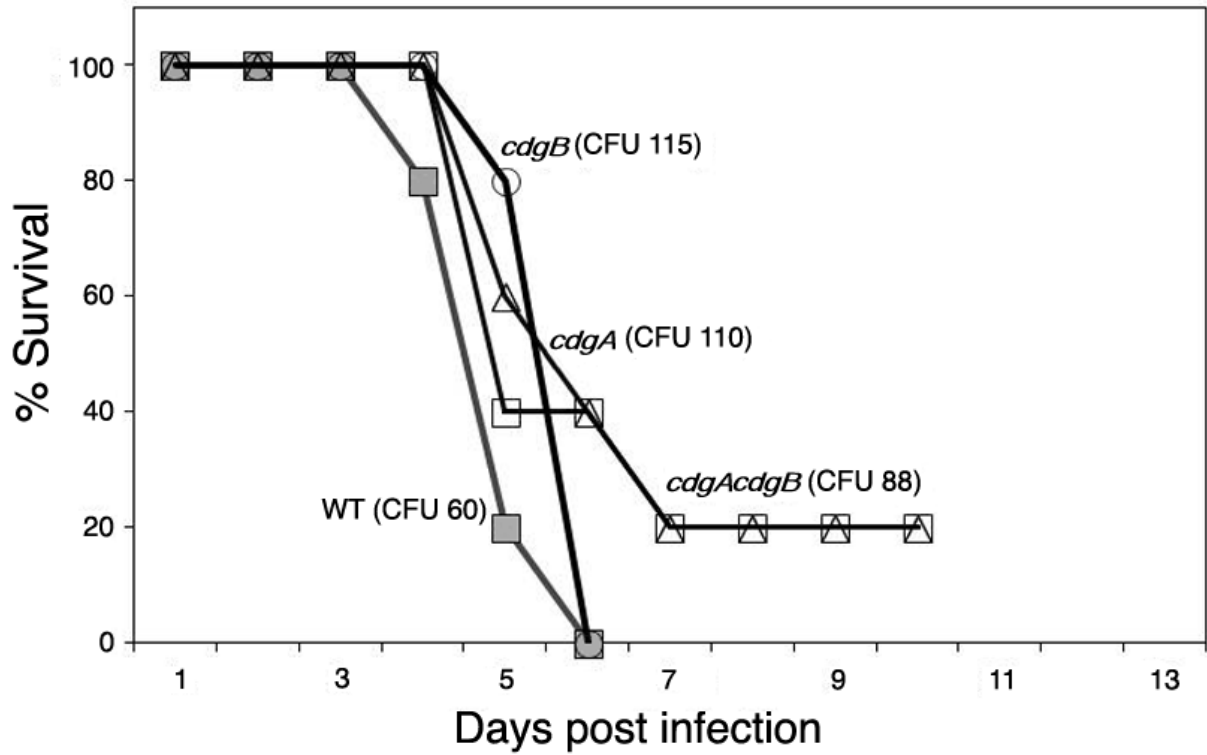


Figure S7: Low cdGMP levels do not inhibit *F. novicida* virulence. *F. novicida* strains U112 (WT), KKF457 (Δ (FTN0451-FTN0456)), KKF535 (*cdgB*), KKF538 (*cdgA*), and KKF539 (*cdgA cdgB*) were inoculated intranasally into groups of 5 female BALB/C mice at the indicated inocula and survival monitored.

Supplemental References:

1. **Anthony, L. S., M. Z. Gu, S. C. Cowley, W. W. Leung, and F. E. Nano.** 1991. Transformation and allelic replacement in *Francisella* spp. *J Gen Microbiol* **137**:2697-2703.
2. **Barker, J. R., A. Chong, T. D. Wehrly, J.-J. Yu, S. A. Rodriguez, J. Liu, J. Celli, B. P. Arulanandam, and K. E. Klose.** 2009. The *Francisella tularensis* pathogenicity island encodes a secretion system that is required for phagosome escape and virulence. *Molecular microbiology* **74**:1459-1470.