Control of Gene Expression in Leptospira spp. by Transcription Activator-Like Effectors Demonstrates a Potential Role for LigA and LigB in Leptospira interrogans Virulence

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Control of gene expression in *Leptospira* spp. by Transcription Activator-Like Effectors (TALEs) demonstrates a potential role for LigA and LigB in virulence in *L. interrogans*.

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Abstract:

Leptospirosis is a zoonotic disease that affects approximately one million people annually with a mortality rate above ten percent. Currently, there is an absence of effective genetic manipulation tools for targeted mutagenesis in pathogenic leptospires. Transcription Activator-Like Effectors (TALEs) are a recently described group of repressors which modify transcriptional activity in prokaryotic and eukaryotic cells by directly binding to a targeted sequence within the host genome. To determine the applicability of TALE within *Leptospira* spp., two TALE constructs were designed. First, a constitutively expressed *tale* specific for the *lacO*–like region upstream of *bgaL* was trans inserted in the saprophyte *Leptospira biflexa* ($TALE_{\beta gal}$). RT-PCR analysis and enzymatic assays demonstrated BgaL was not expressed in $TALE_{\beta gal}$. Second, to study the role of LigA and LigB in pathogenesis, a constitutively expressed *tale* with specificity for the homologous promoter regions of *ligA* and *ligB* was cis inserted into the pathogen *Leptospira interrogans* ($TALE_{lig}$). LigA and LigB expression were studied using three independent clones: $TALE_{lig1}$, $TALE_{lig2}$, and $TALE_{lig3}$. Immunoblot analysis of osmotically induced $TALE_{lig}$ clones demonstrated a 2 to 9 fold reduction in expression of LigA and LigB, with highest reductions noted in $TALE_{lig1}$ and $TALE_{lig2}$, which were avirulent in vivo and non-recoverable from animal tissues. This study reconfirms galactosidase activity in the saprophyte, and suggests a role for LigA and LigB in pathogenesis. Collectively, this study demonstrates that TALE is effective at reducing expression of targeted genes within saprophytic and pathogenic strains of *Leptospira* spp., providing an additional genetic manipulation tool for this genus.

Introduction

Leptospirosis, a bacterial infection transmitted by animal reservoirs, has emerged to become a major public health concern in much of the developing world. There are more than one million cases of severe leptospirosis reported each year for which mortality is greater than 10% (1). As a spirochete, *Leptospira* spp. which includes the causative agent of leptospirosis, differ considerably from other members of Gram positive and Gram negative bacteria. Progress in our understanding of the general biology and virulence mechanisms of pathogenic *Leptospira* has been slow and difficult. This is mainly due to the lack of adequate and efficient genetic tools (2). Genetic modification of the pathogen are limited primarily to random transposon mutagenesis and there exist only a few examples of mutants obtained by targeted mutagenesis (3). Thus, there is a clear need for additional tools to develop genetic studies in *Leptospira* spp..

The transcription activator-like effector (TALE) family forms a subset of proteins made by *Xanthomonas* bacterial species that are injected into plants to modulate host gene expression, with each effector directly binding a specific DNA target (4, 5). TALEs are composed of three domains: (i) a central repeat domain, with each repeat unit of 34 amino acids specifying one target base via two adjacent amino acids termed repeat-variable diresidues, (ii) an N-terminal translocation signal, and (iii) a C-terminal region containing a transcriptional activation domain as well as a nuclear localization signal. These DNA binding proteins can be engineered to target novel DNA sequences (6). Transcriptional repression by TALEs is a common approach to control gene expression in higher organisms as diverse as plants, fruit flies, zebrafish, mice, and human cell lines. More recently, the TALE genetic system has been imported in *Escherichia coli* (7). Here, we imported this new genetic system based on TALE, to allow targeted repression of gene expression in both the saprophyte *L. biflexa* and the pathogen *L. interrogans*.

Materials and Methods

Strains and culture conditions

The following *Leptospira* strains were used in this study: pathogen *Leptospira interrogans* serovar Manilae strain L495, and saprophyte *Leptospira biflexa* serovar Patoc strain Patoc1. *E. coli* XL-10 gold ultracompetent cells (Agilent, Santa Clara, CA) were used for construction of *flgB-tale* constructs, and *E. coli* strain S17.1 (8) was used for conjugation. Leptospires were grown at 30˚C on a rotary shaker in Ellinghausen-McCullough-Johnson-Harris (EMJH) liquid media as previously described (9, 10). *Leptospira* transformants were initially isolated on solid EMJH media with 40 µg/ ml spectinomycin (Sigma-Aldrich Chimie, Saint-Quentin-Fallavier, France) as previously described (11). *E. coli* cells were grown in Luria broth or Luria solid media supplemented with ampicillin (100 µg / ml), kanamycin (50 µg /ml) and spectinomycin (50 µg / ml) when required. For NaCl induced *Leptospira* cultures, strains were grown at 30°C to late exponential phase in 50 ml of liquid EMJH with a final concentration of 120 mM NaCl.

Bioinformatic analyses

Bioinformatic analyses were completed by Microscope Microbial Genome Annotation & Analysis Platform web based software (https://www.genoscope.cns.fr/agc/mage) (12). LigA, LigB, and FlaA2 expression were quantitated using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014) which is made available by the United States National Institutes of Health (Bethesda, MD).

TALE construct design

The custom TALE genes used in this study were synthesized by GeneArt (Life Technologies, Grand Island, NY, USA). The genes (2523 bp), which include 5' NdeI and 3'

XbaI restriction sites, were codon-optimized for expression in *Leptospira* spp. The availability of codon usage tables for the genomes *of L. interrogans* strains allowed us to define preferred and rare codons. The TALE constructs were then manually codon-optimized by using codons frequently used in *L. interrogans*.

Based on this methodology, a TALE specific for the *lacO*-like region of *L. biflexa*, named $tale_{\beta gal}$, was designed to anneal at sequence 5'-AAGATAGATTCTGCAATCG-3'. The $tale_{\beta gal}$ gene was digested with NdeI and XbaI, purified, and inserted between the NdeI and SpeI restriction sites of pCRPromFlgB (13) to generate a transcriptional fusion between the *tale* gene and the *Borrelia burgdorferi flgB* promoter. The DNA fragment containing the transcriptional fusions was released by PvuII digestion and cloned into the SmaI site of the *E. coli-L. biflexa* shuttle vector pMAT (14) to generate the plasmid p$tale_{\beta gal,}$ which replicates in *L. biflexa*. Another TALE construct, named $tale_{lig}$, was designed to anneal to the sequence 5'-TCCAATAAATCTTAAGAGA-3' which is located in homologous promoter regions of *ligA* and *ligB* in *L. interrogans* 170 basepairs upstream of +1 (15). The $tale_{lig}$ gene was inserted downstream of the *B. burgdorferi flgB* promoter (as described above) and the DNA fragment containing the fusion was released by ApaI and KpnI digestions (Thermo Scientific, Waltham MA), gel purified (Qiagen, Venlo, Netherlands), and subcloned into the corresponding sites of pAL614 (16). Plasmid constructs (p$tale_{lig}$) were introduced into *Leptospira* strains by conjugation with *E. coli*, as described previously (17). Spectinomycin-resistant clones were confirmed by PCR amplification of *aadA* (primers spcA/spcB) and $tale_{lig}$ (primers TA5F/TA5R) and *flgB*-$tale_{lig}$ (pFLGA/TA5R) (table1). The position of $tale_{lig}$ cis insertion in *Leptospira interrogans* transformants were determined as previously described (18) using primers TnSp1/Deg1 or TnSp1/Deg2 (for initial amplification) followed by primers 3855/Tag

126 (for nested PCR) (table 1). DNA sequencing was completed by Eurofins Genomics
127 (Ebersberg, Germany).

128 **Reverse Transcriptase PCR**

129 For RT-PCR experiments, 8 mL of cells were grown at 30˚C on a rotary shaker to late
130 logarithmic growth in EMJH liquid media. Cells were collected by centrifugation for 15
131 minutes at 4000 x g. Pelleted cells were re-suspended in 700 µl of TRIzol and RNA was
132 purified according to manufacturer's instructions (Invitrogen, Saint Aubin, Ile de France,
133 France). DNA was degraded using Turbo DNA-free kit according to manufacturer's
134 instructions (Invitrogen). 1 µg of DNAse-treated RNA per sample was used to generate
135 cDNA with iScript cDNA synthesis kit as per manufacturer's instructions (Bio-Rad,
136 Marnes-la-Coquette, France). PCR amplifications were performed to determine the
137 presence of cDNA for $tale_{\beta gal}$ using primers TAF5/TA5R (table 1), and *bgaL* using
138 primers LEPBla0024F/LEPBla0024R (table 1).

139 **β-galactosidase assay**

140 A β-galactosidase colorimetric assay adapted from Miller (19) was used for
141 determination of β-galactosidase activity in *L. biflexa*. The cell concentration of
142 leptospire cells in logarithmic growth was determined by Petroff-Hausser counting
143 chamber according to manufacturer's instructions (Hausser Scientific, Horsham PA). 3 x
144 10^8 cells per sample were centrifuged at 6700 cfm for 10 minutes to pellet cells. The
145 supernatant of each sample was discarded, and cells were re-suspended in 1 ml of Z
146 buffer pH7 (130 mM monosodium phosphate, 40 mM disodium phosphate, 10 mM
147 potassium chloride, 2 mM magnesium sulfate, 40 mM β-mercaptoethanol). Optical
148 density of each culture was measured by spectrophotometry (OD_{420}). 20 µl of
149 chloroform (final volume 2% v/v) and 20 µl of 0.1% SDS (final volume 0.02% v/v) was
150 added to each re-suspended sample. The sample was vortexed for 10 seconds, and then

samples were placed at 30°C for 5 minutes. Following incubation, 200 µl of a 13.27 mM solution of o-nitrophenyl-B-D-galactopyranoside (ONPG, 4 mg/ mL) re-suspended in Z buffer was added to samples. Samples were gently rocked and observed until samples turned yellow, or for 24 hours if no color change was observed. The reaction was stopped by adding 248 µl of sample with 100 µl of 1M sodium carbonate to samples. Color change was measured by spectrophotometric analyses at OD_{420} and OD_{550}. To calculate Miller units (mU), the following formula was used: $mU=1000(OD_{420}-(1.75 \times OD_{550}))/$ (time in minutes x volume of culture in mL x initial OD_{420} of culture).

Blue-White Screening

β-galactosidase activity was also determined by blue-white screening of *L. biflexa* colonies. *L. biflexa* colonies were grown on 20 ml of solid EMJH media. After colony formation, approximately 0.04 ml of 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside in DMSO (X-gal, 40 mg/ml) was added to plates. Plates were incubated overnight at 30˚C before photographs were taken. Colonies of wild type (n=910), βgal^- (n=221) and $TALE_{\beta gal}$ (n=557) were counted from at least 4 independent plates. Percentage of blue versus white colonies was calculated, and the average and standard error mean were reported. Statistical analysis was performed by one-way analysis of variance.

Immunoblot analysis

Cells were grown to late logarithmic phase under physiological osmotic shock conditions (120 mM NaCl in EMJH liquid medium). Cells were pelleted, washed in phosphate-buffered saline (PBS), resuspended in 0.2 mL of lysis buffer (25 mM Tris-HCl, 2 mM EDTA, 150 mM KCl, 5mM DTT in ddH_2O, pH 7.5), and sonicated. For the representative membrane shown, 3.5 µg of protein per sample was used for SDS-PAGE and PVDF membrane transfer according to manufacturer's instructions (Bio-Rad).

Immunoblots were performed as previously described (20) with the following primary antibodies: anti-LigA/B (1:1000 dilution), or anti-FlaA2 (1:2000 diluted in blocking buffer). Membranes were probed with goat anti-rabbit IgG-HRP conjugated antibody (1:100,000 dilution) according to manufacturer's instructions (Sigma-Aldrich Chimie). Chemiluminescent detection was performed with Supersignal West Pico Chemiluminescent substrate according to manufacturer's instructions (Life Technologies). The mean and SEM of three independent replicates per transformant and wild type were used to calculate the mean and SEM of LigA/LigB fold reduction in TALE$_{lig}$ transformants compared to wild type. Statistical analysis was performed by one-way analysis of variance.

Virulence assay

Low passage leptospires were grown to mid-late logarithmic phase. Cell concentration was quantitated by Petroff-Hausser counting chamber as described above. Cells were diluted to 10^6 cells/ mL in EMJH medium. Four week old male Golden Syrian Hamsters (*Mesocricetus auratus*, RJHan:AURA) were purchased from Janvier Labs (Saint-Berthevin, France). Four hamsters per group were inoculated via intraperitoneal injection with 1 mL of 10^6 cells. The animals were observed daily for symptoms of leptospirosis. The animals were euthanized if symptoms of leptospirosis were present, or at 21 days post inoculation in absence of disease manifestation as previously described (19). Kidney tissue and blood were collected from euthanized animals, homogenized under sterile conditions in 5 mL of EMJH media, and incubated at 30°C for 4 weeks to recover viable spirochetes. Statistical analysis of survival data was performed by the Log-rank (Mantel-Cox) test. Protocols for animal experiments conformed to the guidelines of the Animal Care and Use Committees of the Institut Pasteur (Paris, France).

Results and Discussion

tale$_{\beta gal}$ represses β-galactosidase activity in *L. biflexa*.

A putative DNA-binding site for the lactose repressor, also called the lac operator or *lacO*, which is homologous to the one characterized in *E. coli* (5'-AATTGTGAGCGCTCACAATT-3') was identified downstream of the Shine-Dalgarno sequence within the promoter region of *LEPBIa0024* (*bgaL*) encoding for a β-galactosidase (21). Accordingly, a *tale* gene was designed for the promoter region 5'-AAGATAGATTCTGCAATCG-3' (NT 24090-24107) which is located 14 basepairs upstream of the +1 site of *bgaL* and named *tale$_{\beta gal.}$* *tale$_{Bgal}$* was cloned into the replicative vector pMAT, then transferred into *L. biflexa* as described above. The transformant was named TALE$_{\beta gal}$. RT-PCR verified that *tale$_{\beta gal}$* is expressed by TALE$_{\beta gal}$ in *L. biflexa* (fig. 1A). Amplification of *tale* from TALE$_{\beta gal}$ also serves as a positive control that RNA was purified, and that cDNA was generated from TALE$_{\beta gal}$. Further, *tale$_{\beta gal}$* abrogated transcription of *bgaL* in TALE$_{\beta gal}$ (fig. 1A). To confirm that *bgaL* repression by *tale$_{\beta gal}$* results in abrogation of β-galactosidase activity in the transformant TALE$_{\beta gal}$, enzymatic activity analysis was completed comparing β-galactosidase activity in wild type to *βgal$^-$* (a spontaneous *L. biflexa* frameshift mutant at NT24353), and TALE$_{\beta gal}$. First, strains were tested by blue-white colony screening to determine if they were capable of cleaving X-gal. Colonies grown on EMJH solid media were treated with 80 μg/ ml of X-gal and left for 16 hours at 30°C. Wild type was capable of β-galactosidase activity, hydrolysing X-gal into galactose and 4-chloro-3-brom-indigo, creating a characteristic blue colony in 97.6%±1.7 of colonies. All *βgal$^-$* (100%±0) and TALE$_{\beta gal}$ (100%±0) colonies remained white after incubation with X-gal (fig. 1B), which were statistically significant compared to wild type ($P<0.01$, and $P<0.01$, respectively). Additionally, a modified Miller colorimetric assay (19) was used to measure β-galactosidase activity as described above. Wild type exhibited 1.43 mU of

activity, while *βgal*$^{-}$ and TALE$_{βgal}$ both exhibited 0 mU of activity. Together, this data demonstrates that TALE is an effective modulator of expression in the saprophyte *L. biflexa*, and concurs with previous data that *bgaL* has β-galactosidase activity (21).

TALE$_{lig}$ reduces expression of LigA and LigB in *L. interrogans*

A *tale* specific for the leptospiral immunoglobin-like (lig) genes *ligA* and *ligB* was designed to anneal to 5'- TCCAATAAATCTTAAGAGA -3'. This sequence corresponds to the -10 promoter region, which is a homologous region 170 basepairs upstream of +1 of *ligA* (*lmanv2_30028*) and *ligB* (*lmanv2_350013*) (15). Three biologically distinct TALE$_{lig}$ transformants were characterized: TALE$_{lig1}$, TALE$_{lig2}$, TALE$_{lig3}$. Since insertion is due to random insertion by the transposon *Himar1*, sequence analysis by nested PCR using degenerate primers was completed to determine *tale$_{lig}$* insertion position in each transformant. *tale$_{lig}$* inserted in TALE$_{lig1}$ at position NT818455 within the proximal region of *lmanv2_90191* (NT 818032-819462, putative polysaccharide deacetylase), *tale$_{lig}$* inserted in TALE$_{lig2}$ at NT 1270962 within the proximal region of *lmanv2_150048,* (NT 1270809-1271918, putative *pilF*), and *tale$_{lig}$* inserted in TALE$_{lig3}$ at the distal region of *lmanv2_340018* (NT 2894815-2896014, putative conserved membrane protein of unknown function).

TALE$_{lig}$ transformants were grown in physiological osmotic conditions in vitro, and expression of LigA and LigB was determined by immunoblot analysis. This study focused on immunoblot analysis, as these data are more informative of the ability of *tale$_{lig}$* to repress expression at the protein level within Tale$_{lig}$ transformants, which was a pre-requisite for in vivo experimentation. Expression of Lig proteins were normalized to expression of FlaA2 per sample. TALE$_{lig1}$ had a 3.1±1.2 fold reduction of LigA ($P<0.05$), and a 6.6±1.2 fold reduction of LigB ($P<0.05$) compared to wild type. TALE$_{lig2}$,

had a 5.3±1.3 fold reduction of LigA ($P<0.05$), and a 9.1±3.8 fold reduction of LigB ($P<0.05$) compared to wild type. TALE$_{lig3}$ had a of 2.1±0.4 fold reduction of LigA ($P<0.05$), and a 4.6±1.6 fold reduction of LigB compared to wild type ($P<0.05$) (fig. 2A). The variability in *ligA* and *ligB* repression amongst transformants may suggest that expression of *tale*$_{lig}$ (and concordant repression of *ligA* and *ligB*) might be affected by the chromosomal location of Tn-*tale*$_{lig}$. Since the TALE system currently relies on random insertion by the Himar1 transposon system, it is suggested that multiple transformants should be selected and screened when using the TALE system in the pathogen.

Repression of LigA and LigB may suggest a role of virulence of *L. interrogans*

Hamsters were inoculated intraperitoneally with 10^6 cells of wild type, TALE$_{lig1}$, TALE$_{lig2}$, or TALE$_{lig3}$ transformants and observed for leptospirosis for 21 days. TALE$_{lig3}$ transformant had reduced virulence compared to wild type ($P=0.09$), while TALE$_{lig1}$ and TALE$_{lig2}$ transformants were avirulent compared to wild type ($P<0.01$, $P<0.01$, respectively) (fig. 2B). Tissue samples from hamsters were harvested following euthanasia to attempt recovery of viable spirochetes. Motile leptospires were recovered from wild type and TALE$_{lig3}$ transformant infected animals, however no spirochetes were recovered nor observed from tissues harvested from animals infected by neither TALE$_{lig1}$ nor TALE$_{lig2}$ transformants. LigA and LigB protein expression differences between TALE$_{lig}$ transformants may explain their different virulence profiles. Alternatively, it remains possible that attenuation of virulence of TALE$_{lig}$ transformants is the result of chromosomal insertion of Tn-*tale*$_{lig}$ and the subsequent inactivation of these disrupted genes in the TALE$_{lig}$ transformants that were studied. Therefore, future

work to determine the role of *lmanv2_90191*, *lmanv2_150048* (*pilF*), and *lmanv2_340018* is warranted.

Previous research has shown that LigA and LigB are surface exposed proteins (22), and that they play a role in adhesion of spirochetes to extracellular matrix proteins, complement regulators, and animal cells (15, 23, 24). Further, single gene mutants of either *ligA* in *L. interrogans* serovar Manilae strain L495 or *ligB* in *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 did not reduce virulence in standard animal models, and remained recoverable from animal tissue (25, 26). The distribution and sequences of *lig* genes, including *ligA*, *ligB*, and *ligC*, (27, 28) as well as the level of expression of Lig proteins (22) may vary in each pathogenic serovar. Depending on the strain, inactivation of only one *lig* gene may therefore be compensated by the expression of another Lig protein(s). The status of the *lig* genes in the *L. interrogans* serovar Manilae remains to be clarified.

Previous analyses have demonstrated homology in the N-terminal repeat domains of LigA and LigB (encompassing 266 bp upstream of their start codon until 1890 bp into *ligA* and *ligB*) with variation at the C-terminal region of LigB (22). As has been previously suggested, LigA and LigB may work as a redundant system to ensure spirochete adhesion and survival within a mammalian host (25, 29). As the current data suggests, reduced expression of both LigA and LigB proteins within the TALE$_{lig}$ transformants conferred reduced virulence within hamsters, which may suggest a cumulative role of LigA and LigB in pathogenesis.

In conclusion, the current study demonstrates that the TALE system is an efficient system of targeted gene repression in saprophytic and pathogenic leptospires, and suggests a role of Lig proteins in pathogenesis. TALEs act as DNA-binding repressors by inhibiting promoter recognition by RNA polymerase or blocking transcription; however,

the promoter regions of *Leptospira* are poorly characterized at present. Insight into the promoter motif of leptospires allows increased efficiency of the TALE system, as demonstrated here by TALE$_{\beta gal}$. It is therefore suggested that future research focused on determining these promoter motifs and transcriptional start sites will aid in designing TALEs exhibiting tight repression of gene expression in *Leptospira* spp.

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406 **Figure legends**

407

408 **Figure 1:** TALE$_{\beta gal}$ represses β-galactosidase activity in *L. biflexa.*

409 **A.** RT-PCR of *L. biflexa* wild type (WT) and TALE$_{\beta gal}$. Left image shows that TALE$_{\beta gal}$
410 transcribes *tale*$_{\beta gal}$. Right image shows that WT transcribes *bgaL*, while TALE$_{\beta gal}$ does
411 not.

412 **B.** Wild type, natural mutant Patoc *βgal*$^{-}$, and TALE$_{\beta gal}$ were grown on solid phase EMJH
413 and exposed to X-gal for 16 hours. Wild type was able to cleave X-gal to galactose and 5-
414 bromo-4-chloro-3-hydroxyindole, creating a blue colour. Natural mutant Patoc *βgal*$^{-}$ and
415 TALE$_{\beta gal}$ were unable to cleave X-gal; thus the colonies remained colourless.

416

417 **Figure 2:** TALE$_{lig}$ represses expression of LigA and LigB in *L. interrogans*.

418 **A.** A representative immunoblot of *L. interrogans* wild type (WT) and 3 biologically
419 distinct TALE$_{lig}$ transformants (TALE$_{lig1}$, TALE$_{lig2}$, TALE$_{lig3}$). 3.5 μg of protein extract/
420 sample was reacted with antisera specific for LigA/B (top images) or FlaA2 (bottom
421 images). Analysis revealed a 3.1±1.2 fold reduction of LigA ($P<0.05$), and a 6.6±1.2 fold
422 reduction of LigB ($P<0.05$) in TALE$_{lig1}$ compared to WT; a 5.3±1.3 fold reduction of LigA
423 ($P<0.05$), and a 9.1±3.8 fold reduction of LigB ($P<0.05$) in TALE$_{lig2}$ compared to WT; a
424 2.1±0.4 fold reduction of LigA ($P<0.05$), and a 4.6±1.6 fold reduction of LigB ($P<0.05$) in
425 TALE$_{lig3}$ compared to WT.

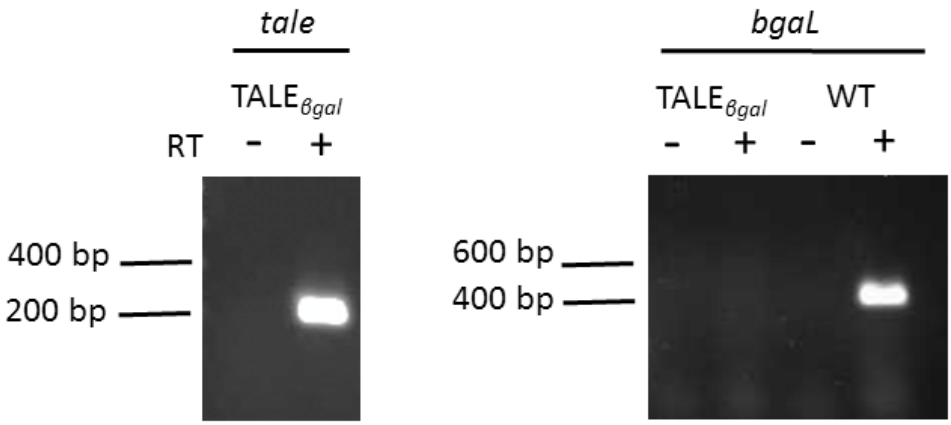
426 **B**. Survival analysis of intraperitoneal inoculation of 10^6 cells of WT, TALE$_{lig1}$, TALE$_{lig2}$,
427 or TALE$_{lig3}$ in hamsters showed reduced virulence of TALE$_{lig3}$ compared to wild type
428 ($P=0.09$) and significant attenuation of virulence in TALE$_{lig1}$ ($P<0.01$) and TALE$_{lig2}$
429 ($P<0.01$) compared to wild type.

430

431 Table 1: Primers used in this study

Primer Name	Sequence (5' → 3')	Expected Size	Target
TA5F	CATATGGTTGATCTTCGTAC	264 bp	amplification of proximal region of *tale*
TA5R	AAGTGCACGTGCACCTGACC		
LEPBIa0024F	AATATAAAACTCTCGATTCGCTTAACA	500 bp	amplify internal region of *L. biflexa bgaL*
LEPBIa0024R	TCACAATTGCTTGTGTCAACC		
spcA	GGGGTGAATTTGAGAATGGA	246 bp	*aadA* cassette
spcB	GTCACTGTTTGCCACATTCG		
tnsp1	GAAACTTCTCTCAATTAGG		spc^R transposon
Deg1	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT	varies	Degenerate oligo with 5' tag
Deg2	GGCCACGCGTCGACTAGTACNNNNNNNNNNTCTT		Degenerate oligo with 5' tag
3855	TTCTTGACGAGTTCTTCTGA	varies	nested primer
tag	GGCCACGCGTCGACTAGTAC		binds to tag on Deg1/ Deg2
pFLGA	TAATACCCGAGCTTCAAGGAAG	671 bp	*flgB-tale*
TA5R	AAGTGCACGTGCACCTGACC		

A


tale
TALE$_{\beta gal}$
RT - +
400 bp
200 bp
bgaL
TALE$_{\beta gal}$ WT
- + - +
600 bp
400 bp

B

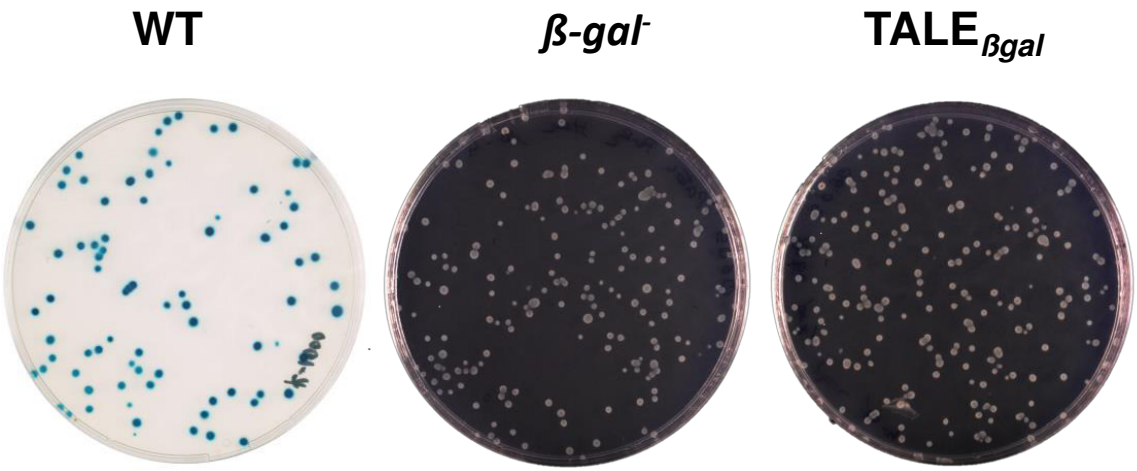
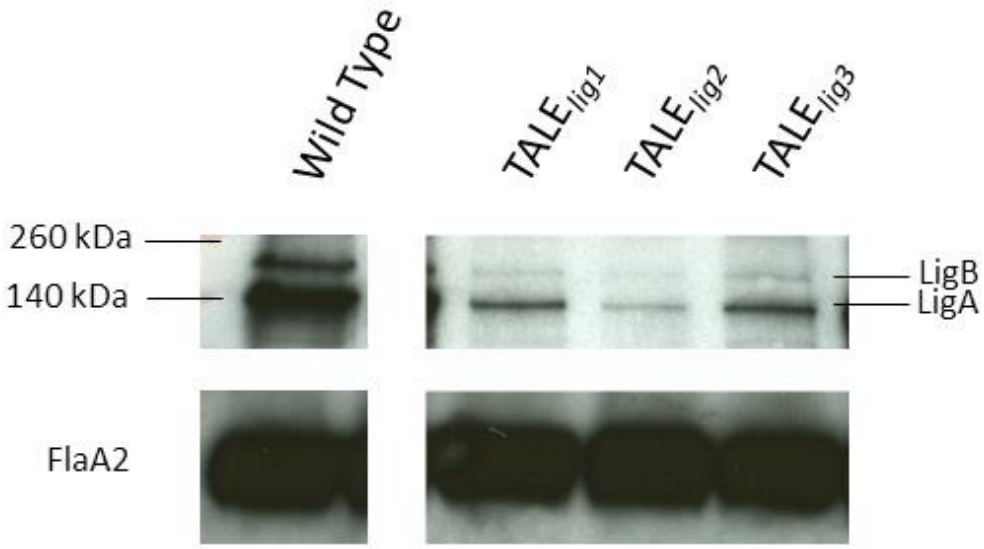

WT
ß-gal$^-$
TALE$_{\beta gal}$

Figure 1

A


Wild Type
TALE lig1
TALE lig2
TALE lig3
260 kDa
140 kDa
LigB
LigA
FlaA2

B

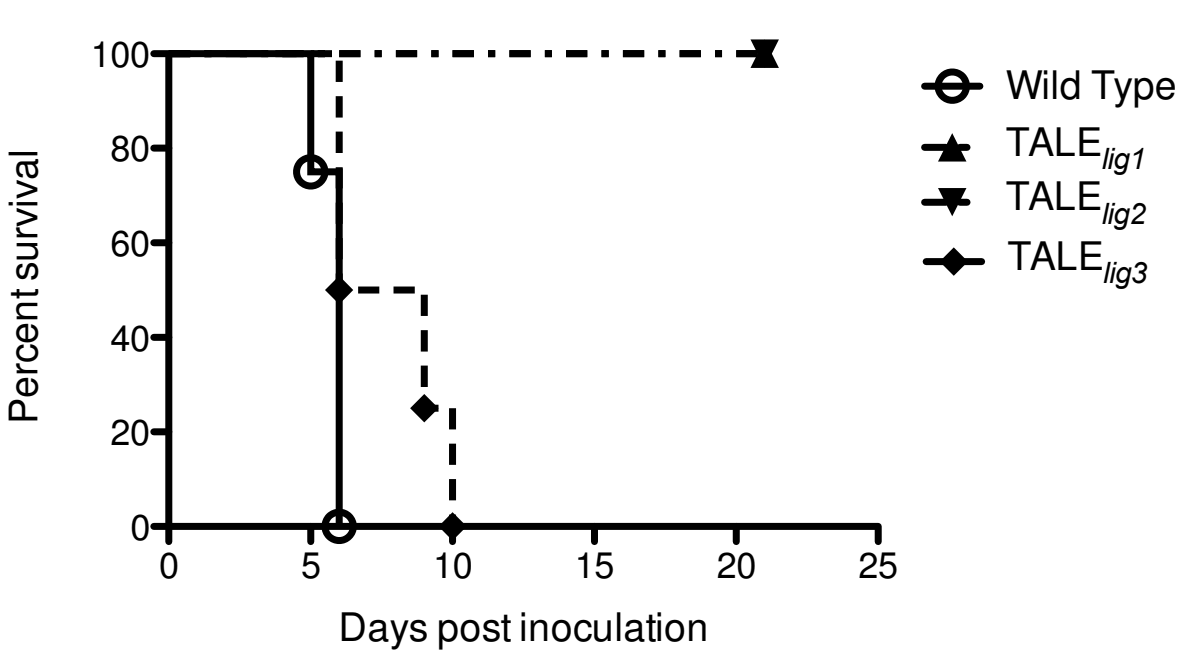

Percent survival
100
80
60
40
20
0
0
5
10
15
20
25
Days post inoculation
Wild Type
TALE lig1
TALE lig2
TALE lig3

Figure 2