Supporting Information

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SI Materials and Methods

Bacterial Strains. *Campylobacter jejuni* 81–176 *rpsL*^{Sm} mutant strains previously described include Δ*rpoN* (DRH321) (1) and Δ*astA* (DRH461) (2). Previously described *C. jejuni* 81–176 *rpsL*^{Sm} Δ*astA* mutant strains include Δ*rpoN* (DRH453) (2); Δ*flgR* (DRH749) (2); Δ*flgS* (DRH911) (2); Δ*flhA* (DRH979) (2); *flgR*$_{\Delta CTD}$ (DRH1931) (3); Δ*flgS flgR*$_{\Delta CTD}$ (SNJ227) (3); Δ*flhA flgR*$_{\Delta CTD}$ (SNJ235) (4); and Δ*flgS flgR D51A*$_{\Delta CTD}$ (SNJ713) (3). *Escherichia coli* DH5α was used for all cloning procedures. Electroporation of *C. jejuni* and creation of insertional and in-frame deletions was performed by previously published protocols (1, 5).

Bacterial Growth Conditions. For all experiments, *C. jejuni* strains were initially grown from freezer stocks on Mueller–Hinton (MH) agar containing 10 μg/mL trimethoprim for 48 h under microaerobic conditions at 37 °C. *Campylobacter* defined media (CDM) contains nutrients at concentrations to support growth (6). When appropriate, sodium pyruvate was added to CDM at 50 mM and 100 mM excess. After initial growth, strains were restreaked onto appropriate media and grown for another 16 h for use in experiments. Chloramphenicol and kanamycin were added to media at 10 and 50 μg/mL, respectively, when necessary.

Construction of *C. jejuni* Mutants and Transcriptional Reporter Strains. The *pta ackA* locus was amplified with 750 bp of flanking sequence by PCR from *C. jejuni* 81–176 chromosomal DNA with primers containing 5′ KpnI sites. After cloning into KpnI-digested pUC19 (creating pJMB553), a SmaI-digested *cat-rpsL* cassette (from pDRH265) (1) was cloned into the AflII site in *ackA* (generating pJMB653) or the SpeI site in *pta* (creating pJMB565). Interruption of both *pta* and *ackA* was accomplished by inserting SmaI-digested *cat-rpsL* cassette in AflII- and SpeI-digested pJMB553 (creating pJMB955). PCR-mediated mutagenesis was performed with pJMB553 to fuse the start and stop codons, thereby deleting the remainder of the coding sequence of *ackA* and creating pJMB630.

Plasmids pJMB653, pJMB565, and pJMB955 were introduced into *C. jejuni* 81–176 *rpsL*^{Sm} strains Δ*astA* Δ*flgS* (DRH911), Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ (SNJ227), and Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ (SNJ713) by electroporation, generating 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS ackA::cat-rpsL* (JMB740), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta::cat-rpsL* (JMB1066), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta ackA::cat-rpsL* (JMB971), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *ackA::cat-rpsL* (JMB865), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta::cat-rpsL* (JMB862), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta ackA::cat-rpsL* (JMB977), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *ackA::cat-rpsL* (JMB732), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta::cat-rpsL* (JMB825), and 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta ackA::cat-rpsL* (JMB974). Additionally, pJMB653 was electroporated into 81–176 *rpsL*^{Sm} Δ*astA* (DRH461) and 81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ (DRH1931) to generate 81–176 *rpsL*^{Sm} Δ*astA ackA::cat-rpsL* (JMB669) and 81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ *ackA::cat-rpsL* (JMB811). Mutants were recovered on MH agar containing chloramphenicol and verified by colony PCR. Semiquantitative real-time RT-PCR was used to ensure that the *pta*::*cat-rpsL* mutation did not cause a polar effect on transcription of the downstream *ackA* gene.

C. jejuni 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS* Δ*ackA* (JMB760), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ Δ*ackA* (JMB919), 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ Δ*ackA* (JMB815), and 81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ Δ*ackA* (JMB857) were created by electroporating JMB740, JMB865, JMB732, and JMB811 with pJMB630. Transformants were recovered on MH agar containing 0.5–5 mg/mL streptomycin. Deletion of *ackA* was verified by colony PCR.

To construct strains containing chromosomal promoterless *astA* transcriptional reporters, pDRH610 (containing *flaB*::*astA-kan*) (2) or pDRH669 (containing *flgDE2*::*astA-kan*) was electroporated into all relevant strains to replace native *flaB* or *flgDE2* on the chromosome with the *flaB*::*astA* or *flgDE2*::*astA* transcriptional fusion. Mutants were recovered on MH agar containing kanamycin and verified using colony PCR.

Construction of Plasmids Containing *flgDE2*::*astA* Transcriptional Fusions. An *astA-kan* cassette was released from pDRH580 by digestion with SmaI and cloned into the MscI site of *flgD* in the *flgDE2* locus of pDRH351 (2). One plasmid (pDRH669) was recovered with *astA-kan* in the proper orientation to create a *flgD*::*astA* transcriptional fusion, with *astA-kan* located 582 bp downstream of the *flgDE2* transcriptional start site.

PCR was used to amplify *flgDE2*::*astA* with different 5′ endpoints, including at bases −302, −29, and −13 relative to the start site of transcription of *flgDE2*. Primers were used that contained 5′ PstI sites and 3′ KpnI sites. Transcriptional fusions were cloned into PstI- and KpnI-digested pRY108, an *E. coli*/*C. jejuni* shuttle plasmid (7), to result in pJMB1074 (with a 5′ end at base −302), pJMB1075 (with a 5′ end at base −29), and pJMB1076 (with a 5′ end at base −13). Plasmids were transferred to DH5α/pRK212.1 and then conjugated into *C. jejuni* 81–176 *rpsL*^{Sm} Δ*astA* (DRH461), *C. jejuni* 81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ (DRH1931), *C. jejuni* 81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR* (DRH749), and *C. jejuni* 81–176 *rpsL*^{Sm} Δ*astA* Δ*rpoN* (DRH453). Transconjugants were selected for by growth on MH agar containing 10 μg/mL chloramphenicol, 100 μg/mL streptomycin, and 10 μg/mL trimethoprim.

Semiquantitative Real-Time RT-PCR Analysis. *C. jejuni* strains were grown from frozen stocks on agar containing appropriate antibiotics at 37 °C for 48 h under microaerobic conditions and then restreaked on MH agar and grown for another 16 h. Total RNA was extracted with TRIzol (Invitrogen), and RNA was treated with DNaseI before analysis. RNA for analysis was used at a concentration of 50 ng/μL. Semiquantitative real-time RT-PCR was performed using a 7500 real-time PCR system (Applied Biosystems). Detection of *secD* mRNA served as an endogenous control, and experimental transcript levels were compared with strains DRH461, which served as a WT control in these studies.

Purification of Proteins. Expression of *flgR* constructs for purification of proteins with C-terminal 6XHis tags was performed as previously described (3). Expression of *flgS* with an N-terminal 6XHis tag was performed as described previously (8). For phosphorylation experiments, FlgS and FlgR proteins were purified as described previously (3, 8). For purification of 6XHis-tagged FlgS for phosphorylation of FlgR for EMSAs, cell lysates after production of 6XHis-tagged FlgS were loaded onto a HisTrapFF column (GE Biosciences) for affinity purification. Proteins were eluted using a linear gradient of 250 mM imidazole in 50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM imidazole (pH 8.0). Eluted proteins were dialyzed overnight in 20 mM Tris·HCl (pH 8.0). Dialyzed proteins were loaded onto a HiTrap Q HP column (GE Biosciences) in 20 mM Tris·HCl (pH 8.0) and

eluted by linear gradient with addition of 1 M NaCl. Proteins were dialyzed as described and stored at −80 °C (8).

EMSAs. 6XHis-FlgS and -FlgR proteins were purified as described above. EMSAs were performed based on a modified protocol (9). A 381-bp DNA fragment of *flgDE2* spanning −302 to +79 relative to the transcriptional start site, and a 348-bp DNA fragment of *gyrA* flanking 298 bp upstream and 50 bp downstream of the start codon, were amplified. After FlgS-mediated phosphorylation of FlgR proteins (3), 0.1–1 μM of FlgR proteins were incubated with ^{32}P-labeled DNA at 25 °C for 20 min. For competition experiments, unlabeled P_{flgDE2} and P_{gyrA} were added at 1:1, 5:1, or 10:1 ratios relative to ^{32}P-labeled P_{flgDE2} DNA, and 1 μM of phosphorylated FlgR proteins were used. After electrophoresis, gels were analyzed with a Storm 820 phosphorimager according to manufacturer's instructions (Amersham Biosciences).

***astA* Transcriptional Reporter Assays.** *C. jejuni* Δ*astA* strains were used in transcriptional reporter assays (Table S3). After growth on appropriate agar, arylsulfatase production from *flgDE2*::*astA* or *flaB*::*astA* transcriptional fusions in strains was measured as described previously (2). Each strain was tested in triplicate, and each assay was performed three times.

Transposon Mutagenesis. *C. jejuni* 81–176 Δ*astA* Δ*flgS* $flgR_{\Delta CTD}$ *flaB*::*astA* chromosomal DNA was used in in vitro transposition reactions with the *darkhelmet* Tn as described previously (1, 2, 10). Tn mutants were recovered on MH agar containing chloramphenicol, kanamycin, and 35 μg/mL 5-bromo-4-chloro-3-indolyl sulfate. The site of the Tn insertion in mutants with light blue or white colony phenotypes was determined as described previously (11).

In Vitro FlgR Autophosphorylation Assays with AcP. Ac[^{32}P] was generated as described previously with minor modifications (12). FlgR proteins (50 and 100 pmol) were incubated with 15 μL of Ac[^{32}P]-generating reaction for 20 min at 37 °C. Analysis of FlgR phosphorylation by FlgS was performed as described (3). Proteins were separated by 10% SDS/PAGE without boiling. Gels were analyzed as described.

Transmission Electron Microscopy. After 16 h growth on appropriate agar, strains were prepared for transmission electron microscopy as described (11). Over 220 individual cells for each strain were analyzed for flagellation. Data from two experiments were combined and averaged, and SDs were determined. Bacteria were grouped into one of three categories: two flagella, producing a flagellum at each pole; one flagellum, producing a flagellum only at one pole; or zero flagella, aflagellated.

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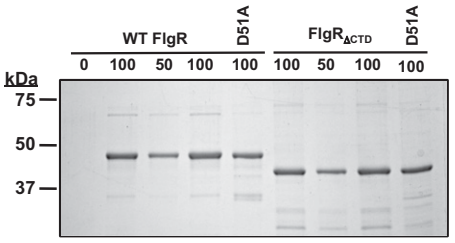

Fig. S1. SDS/PAGE analysis of purified FlgR proteins for autophosphorylation assays. WT FlgR and $FlgR_{\Delta CTD}$ proteins were loaded onto a 10% SDS/PAGE gel identical to that in Fig. 3*A* without preincubation with [^{32}P]ATP or Ac[^{32}P] before electrophoresis. Fifty or 100 pmol of purified WT FlgR or $FlgR_{\Delta CTD}$ or respective D51A mutant proteins are indicated.

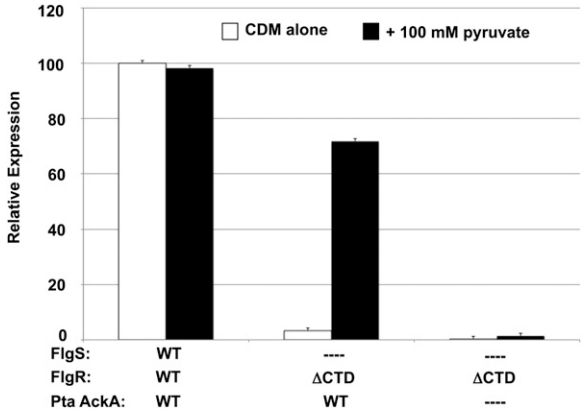

Fig. S2. FlgS-independent activation of $FlgR_{\Delta CTD}$ requires the acetogenesis pathway. Arylsulfatase assay examining *flaB*::*astA* expression after growth on CDM alone (white bars) or CDM with 100 mM sodium pyruvate (black bars). The level of *flaB*::*astA* expression in each strain is relative to WT *C. jejuni* producing the WT FlgSR TCS grown on CDM alone, which was set to 100 units. The FlgS, FlgR, Pta, and AckA proteins produced in each strain are indicated. ΔCTD indicates $FlgR_{\Delta CTD}$. Dashes indicate deletion of respective genes. Error bars indicate SDs.

Table S1. Transcriptional analysis of many σ^{54}-dependent flagellar genes in WT *C. jejuni* and *flgR* mutant strains

	Strain		
Gene*	WT	Δ*flgR*	$flgR_{\Delta CTD}$
flaG	1.00 ± 0.30†	0.13 ± 0.05	2.09 ± 0.66
flgB	1.00 ± 0.05	0.14 ± 0.03	2.19 ± 0.51
flgD	1.00 ± 0.26	0.03 ± 0.01	1.26 ± 0.16
flgE	1.00 ± 0.05	0.02 ± 0.01	1.54 ± 0.15
flgF	1.00 ± 0.10	0.55 ± 0.05	1.88 ± 0.16
flgH	1.00 ± 0.54	0.13 ± 0.06	0.74 ± 0.07
flgI	1.00 ± 0.23	0.24 ± 0.03	2.36 ± 1.05
flgK	1.00 ± 0.27	0.24 ± 0.08	0.96 ± 0.45
fliK	1.00 ± 0.07	0.07 ± 0.04	1.20 ± 0.26

*For genes that are part of flagellar operons, the first gene of the operon shown was analyzed for expression in *C. jejuni* strains.
†The levels of expression of each gene in each strain are relative to WT *C. jejuni*, which was set at 1.0. All analyses were performed in triplicate, and the SDs were calculated.

Table S2. Flagellation of *C. jejuni* mutant strains producing WT FlgS due to AcP-dependent activation of FlgR proteins by altering physiology

		No. of flagella per bacterium*		
Strain	Media	2	1	0
Δ*ackA*	CDM	75 ± 1	22 ± 0	3 ± 1
Δ*ackA*	CDM + pyruvate	75 ± 3	21 ± 4	4 ± 1
$flgR_{\Delta CTD}$	CDM	72 ± 1	24 ± 1	4 ± 0
$flgR_{\Delta CTD}$	CDM + pyruvate	74 ± 9	23 ± 10	3 ± 1
$flgR_{\Delta CTD}$ Δ*ackA*	CDM	74 ± 1	23 ± 1	3 ± 0
$flgR_{\Delta CTD}$ Δ*ackA*	CDM + pyruvate	69 ± 1	26 ± 1	5 ± 1

*The percentage of the population producing a single flagellum at both poles (two flagella), a single flagellum at one pole (one flagellum), or no flagella (zero flagella) after growth on CDM or CDM with 100 mM excess sodium pyruvate are indicated. Data are from the average of two experiments ± SD. Analysis of WT *C. jejuni* is indicated in Table 2.

Table S3. Bacterial strains used in this study

Strain	Genotype	Source
DH5α	*E. coli supE44* Δ*lac*U169 (ϕ80*lacZ*ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	Invitrogen
DH5α/RK212.1	DH5α with conjugation transfer element	(13)
81–176	wild-type *C. jejuni* clinical isolate	(14)
DRH212	81–176 *rpsL*^{Sm}	(1)
DRH321	81–176 *rpsL*^{Sm} Δ*rpoN*	(1)
DRH453	81–176 *rpsL*^{Sm} Δ*astA* Δ*rpoN*	(2)
DRH461	81–176 *rpsL*^{Sm} Δ*astA*	(2)
DRH665	81–176 *rpsL*^{Sm} Δ*astA flaB*::*astA-kan*	(2)
DRH749	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR*	(2)
DRH842	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR flaB*::*astA-kan*	(2)
DRH911	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS*	(2)
DRH939	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flaB*::*astA-kan*	(2)
DRH979	81–176 *rpsL*^{Sm} Δ*astA* Δ*flhA*	(2)
DRH1049	81–176 *rpsL*^{Sm} Δ*astA* Δ*flhA flaB*::*astA-kan*	(2)
DRH1931	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$	(3)
JMB669	81–176 *rpsL*^{Sm} Δ*astA ackA*::*cat-rpsL*	This study
JMB732	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *ackA*::*cat-rpsL*	This study
JMB740	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS ackA*::*cat-rpsL*	This study
JMB760	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS* Δ*ackA*	This study
JMB811	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ *ackA*::*cat-rpsL*	This study
JMB815	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ Δ*ackA*	This study
JMB825	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta*::*cat-rpsL*	This study
JMB842	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS* Δ*ackA flaB*::*astA-kan*	This study
JMB848	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ Δ*ackA flaB*::*astA-kan*	This study
JMB862	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta*::*cat-rpsL*	This study
JMB857	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ Δ*ackA*	This study
JMB865	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *ackA*::*cat-rpsL*	This study
JMB919	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ Δ*ackA*	This study
JMB927	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ Δ*ackA flaB*::*astA-kan*	This study
JMB971	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta ackA*::*cat-rpsL*	This study
JMB974	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta ackA*::*cat-rpsL*	This study
JMB977	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta ackA*::*cat-rpsL*	This study
JMB1040	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta ackA*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1044	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta ackA*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1045	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta ackA*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1060	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *pta*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1065	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *pta*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1066	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta*::*cat-rpsL*	This study
JMB1081	81–176 *rpsL*^{Sm} Δ*astA*/pJMB1074	This study
JMB1103	81–176 *rpsL*^{Sm} Δ*astA*/pJMB1075	This study
JMB1107	81–176 *rpsL*^{Sm} Δ*astA*/pJMB1076	This study
JMB1111	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR*/pJMB1074	This study
JMB1116	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR*/pJMB1075	This study
JMB1118	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgR*/pJMB1076	This study
JMB1124	81–176 *rpsL*^{Sm} Δ*astA* Δ*rpoN*/pJMB1074	This study
JMB1126	81–176 *rpsL*^{Sm} Δ*astA* Δ*rpoN*/pJMB1075	This study
JMB1129	81–176 *rpsL*^{Sm} Δ*astA* Δ*rpoN*/pJMB1076	This study
JMB1136	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$/pJMB1074	This study
JMB1138	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$/pJMB1075	This study
JMB1141	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$/pJMB1076	This study
JMB1154	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS pta*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1280	81–176 *rpsL*^{Sm} Δ*astA ackA*::*cat-rpsL flaB*::*astA-kan*	This study
JMB1302	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ Δ*ackA flaB*::*astA-kan*	This study
SNJ138	81–176 *rpsL*^{Sm} Δ*astA flgR*$_{\Delta CTD}$ *flaB*::*astA-kan*	(3)
SNJ227	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$	(3)
SNJ235	81–176 *rpsL*^{Sm} Δ*astA* Δ*flhA flgR*$_{\Delta CTD}$	(4)
SNJ268	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR*$_{\Delta CTD}$ *flaB*::*astA-kan*	(3)
SNJ272	81–176 *rpsL*^{Sm} Δ*astA* Δ*flhA flgR*$_{\Delta CTD}$ *flaB*::*astA-kan*	This study
SNJ713	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$	(3)
SNJ731	81–176 *rpsL*^{Sm} Δ*astA* Δ*flgS flgR D51A*$_{\Delta CTD}$ *flaB*::*astA-kan*	This study

Table S4. Plasmids used in this study

Plasmid	Genotype/description	Source
pUC19	Amp^R	New England Biolabs
pRY108	Kan^R; *E. coli-C. jejuni* shuttle vector	(7)
pSpaceball1	Contains the *darkhelmet Tn*	(11)
pDRH265	pUC19::*cat-rpsL*	(1)
pDRH351	pUC19::*flgDE2*	(2)
pDRH580	pUC19::*astA-kan*	(2)
pDRH610	pUC19 containing *flaB*::*astA-kan*	(2)
pDRH664	pUC19::*flhA*	(2)
pDRH669	pDRH351 with *astA-kan* cassette cloned into the MscI site within *flgD* of *flgDE2*	This study
pDRH766	pDRH664 containing Δ*flhA*	(2)
pJMB553	pUC19 with 4.4 kb fragment containing *pta* and *ackA* from 81–176 cloned into the KpnI site	This study
pJMB565	pJMB553 with a *cat-rpsL* inserted into the SpeI site within *pta*	This study
pJMB630	pJMB553 with an in-frame deletion of the *ackA* coding sequence fusing start and stop codons	This study
pJMB653	pJMB553 with *cat-rpsL* cloned into the AflII site within *ackA*	This study
pJMB955	pJMB553 *cat-rpsL* cassette cloned into the AflII site within *ackA* and SpeI site within *pta*	This study
pJMB1074	pRY108 with *flgDE2*::*astA* extending to base -302 of P_{flgDE2} cloned into the PstI and KpnI site	This study
pJMB1075	pRY108 with *flgDE2*::*astA* extending to base -29 of P_{flgDE2} cloned into the PstI and KpnI site	This study
pJMB1076	pRY108 with *flgDE2*::*astA* extending to base - 13 of P_{flgDE2} cloned into the PstI and KpnI site	This study