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Supplementary Information for

C*ampylobacter jejuni* BumSR directs a response to butyrate via sensor phosphatase activity to impact transcription and colonization

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Supplementary Materials and Methods

Bacterial strains and plasmids

C. jejuni strains were routinely grown from freezer stocks in microaerobic conditions (10% CO_2, 5% O_2 and 85% N_2) on Mueller-Hinton (MH) agar containing 10 µg/mL trimethoprim at 37 °C for 48 h. Strains were then restreaked onto MH agar with trimethoprim, unless otherwise stated, and grown for an additional 16 h. When appropriate, antibiotics were added to media at the following concentrations: 10 µg/mL chloramphenicol, 100 µg/mL kanamycin, 30 µg/mL cefoperazone, or 0.1, 0.5, 1, 2, or 5 mg/mL streptomycin. *Campylobacter* Defined Media (CDM) contains all 20 amino acids, specific keto acids and other nutrients at concentrations to support growth (1). When needed, CDM is supplemented with 12.5 mM sodium butyrate, 25 mM sodium L-lactate, or 100 mM potassium acetate (all chemicals from Sigma-Aldrich). All CDM media with or without supplementation is buffered to pH 7.0. *E. coli* DH5α, BL21 strains, and XL1-Blue were grown on LB (Lennox L) agar or LB broth containing 100 µg/mL ampicillin, 100 µg/mL kanamycin, 12.5 µg/mL tetracycline, or 15 µg/mL chloramphenicol when necessary. *C. jejuni* strains were stored at -80 °C in a mixture of 85% MH broth and 15% glycerol. *E. coli* strains were stored at -80 °C in a mixture of 80% LB broth and 20% glycerol.

Construction of *C. jejuni* mutants

All bacterial strains and plasmids constructed and used in experiments are listed in Tables S3 and S4. *C. jejuni* mutants were constructed with plasmids purified from *E. coli* that were introduced into *C. jejuni* by electroporation or natural transformation after methylation with EcoRI methyltransferase following previously described methods (2, 3). Cloning of DNA into plasmids was accomplished by standard procedures using T4 DNA ligase or Gibson Assembly Mastermix (New England Biolabs). For cloning of genes to either be deleted from the *C. jejuni* 81-176 *rpsL*^{Sm} (DRH212) or 81-176 *rpsL*^{Sm} Δ*astA* (DRH461) chromosome, interrupted with antibiotic cassettes, or inserted with the promoterless *astA* gene linked to a kanamycin-resistance cassette, DNA fragments containing approximately 750 bases upstream and downstream of the target gene to be mutated were amplified by PCR using primers containing 5' specific restriction sites (2, 4). For creation of DNA fragments with restriction sites within coding sequences of genes or in-frame deletions of specific genes, primers were designed to create two DNA fragments with the mutations, and then the mutations were joined by PCR and cloned into a specific restriction site of pUC19. All DNA fragments cloned were verified by sequencing to result in: pPML849 (pUC19::*peb3*), pKNG327 (pUC19::*bumR*$_{\Delta KFK}$), pKNG210 (pUC19::Δ*peb3*), and pKNG537 (pUC19::*Cjj81176_0580*), which contains a mutation to generate an EcoRV restriction site within the coding sequence of *Cjj81176_0580*. A *cat-rpsL* cassette was removed from pDRH265 by SmaI digestion and ligated into the appropriate restriction sites to interrupt each gene in plasmids pPML849, pKNG327, and pPML101 to create pWPK740, pKNG544, and pPML106, respectively (2, 5).

Plasmids pWPK740 and pKNG544 were subsequently electroporated into *C. jejuni* 81-176 *rpsL*^{Sm} (DRH212) to interrupt the respective genes on the *C. jejuni* chromosome with the *cat-rpsL* cassette. pPML106 was electroporated into 81-176 *rpsL*^{Sm} Δ*astA* (DRH461; (4)). Transformants were recovered on MH agar plates containing chloramphenicol, and mutations were confirmed by colony PCR. The recovered mutants included WPK742 (81-176 *rpsL*^{Sm} *peb3*::*cat-rpsL*) KNG558 (81-176 *rpsL*^{Sm} *Cjj81176_0580*::*cat-rpsL*), and PML318 (81-176 *rpsL*^{Sm} Δ*astA bumS*::*cat-rpsL*).

WPK742 and PML318 were then electroporated with pKNG210 (pUC19::Δ*peb3*) and pPML334 (5), respectively, to replace the *cat-rpsL* interrupted genes with constructs containing in-frame

deletion of each gene, thereby removing the gene from the chromosome of *C. jejuni*. Similarly, ABT749 (81-176 *rpsL*^{Sm} *herA*::*cat-rpsL*) was electroporated with pABT745 to create an in-frame deletion of *herA* (6). After methylation of pKNG327, the plasmid was introduced into PML324 by natural transformation to replace *bumR*::*cat-rpsL* with the *bumR*$_{\Delta KFK}$ allele (5). All transformants were recovered on MH agar containing 0.5 to 5 mg/ml streptomycin. Streptomycin-resistant, chloramphenicol-sensitive transformants were screened by colony PCR. Putative *bumR*$_{\Delta KFK}$ mutants were sequenced to verify that the mutant *bumR* allele was contained on the chromosome at the native location. The recovered mutants included PML363 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS*), KNG467 (81-176 *rpsL*^{Sm} Δ*astA bumR*$_{\Delta KFK}$), ABT754 (81-176 *rpsL*^{Sm} Δ*herA*), and KNG248 (81-176 *rpsL*^{Sm} Δ*peb3*).

Construction of plasmids for *in cis* complementation of *C. jejuni*
To create plasmids for *in cis* complementation of Δ*bumS* or Δ*bumR* mutants, a fragment beginning approximately 300 bp upstream of the *bumS* translational start site and ending at the *bumS* stop codon was amplified by PCR from DRH212 chromosomal DNA and was inserted into the BglIII site of pABT1307 (7) via Gibson Assembly to create pKNG636 (pUC19::*rdxA*::*bumS-kan*). To create *in cis bumR* complementation plasmid, a fragment beginning approximately 300 bp upstream of the *bumS* translational start site and ending at the *bumR* stop codon was amplified by PCR from PML363 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS*) chromosomal DNA and inserted into the BglII site of pABT1307 (7) via Gibson Assembly to create pKNG637 (pUC19::*rdxA*::*bumR-kan*).

After methylation of pABT1307, pKNG636, and pKNG637, plasmids were introduced into by natural transformation to interrupt the *rdxA* gene (7, 8). Mutants were recovered on MH agar supplemented with kanamycin and verified by colony PCR to generate strains KNG640 (81-176 *rpsL*^{Sm} Δ*astA rdxA*::*kan*), KNG643 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS rdxA*::*kan*) , KNG646 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS rdxA*::*bumS-kan*), KNG649 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumR rdxA*::*kan*), and KNG652 (81-176 *rpsL*^{Sm} Δ*astA* Δ*bumR rdxA*::*bumR-kan*).

Creation of genetic reporter and screening of transposon mutagenesis.
A SmaI fragment containing a promoterless *astA* gene linked to a kanamycin-resistance cassette (*astA-kan*) from pDRH580 was inserted into BsaBI-digested pPML849 (4). One clone was identified (pPML873) that contained *astA-kan* in the correct orientation to create a transcriptional reporter with the promoter of *peb3* driving expression of the promoterless *astA* gene. pPML873 was then electroporated into 81-176 *rpsL*^{Sm} Δ*astA* to create PML921 (81-176 *rpsL*^{Sm} Δ*astA peb3*::*astA-kan*).

Chromosomal DNA from PML921 was purified and used in *in vitro* transposition with the *darkhelmet* Tn contained in pSpaceball1 as previously described (2, 9, 10). After DNA transposition and repair, transposed DNA were introduced into PML921 by natural transformation (11). Tn mutants were recovered on CDM agar containing chloramphenicol, kanamycin, 12.5 mM sodium butyrate, and 35 µg/ml 5-bromo-4-chloro-3-indolyl sulfate. Putative transposon mutants with increased *peb3*::*astA* expression were identified as dark blue colonies as opposed to the light-blue colony phenotype of WT 81-176 *rpsL*^{Sm} Δ*astA peb3*::*astA-kan*.

After elimination of mutants that contained the *darkhelmet* Tn in the *peb3*::*astA* reporter, putative transposon mutants were examined by arylsulfatase assays to measure the level of expression of *peb3*::*astA* after growth in CDM alone or CDM with 12.5 mM butyrate. Mutants with significant differences in *peb3*::*astA* expression relative to PML921 after growth in the presence or absence of 12.5 mM butyrate were considered defective for butyrate-modulated

gene expression. The site of the Tn insertion in these mutants was determined as previously described (9).

Arylsulfatase transcriptional reporter assays
For creation of the *peb3*::*astA* transcriptional reporter in *C. jejuni* Δ*bumS* and Δ*bumR* mutants, pPML873 was electroporated into PML363 and PML337 and transformants were selected on MH agar with kanamycin (5). Transformants were screened by colony PCR to recover PML908 (81–176 *rpsL*^{Sm} Δ*astA* Δ*bumR peb3*::*astA-kan*) and PML911 (81–176 *rpsL*^{Sm} Δ*astA* Δ*bumS peb3*::*astA-kan*).

Arylsulfatase assays were performed to measure the level of transcription of *peb3*::*astA* transcriptional fusions on the chromosome of *C. jejuni* Δ*astA* strains as previously described (4, 12, 13). Strains for arylsulfatase assays were first grown from freezer stocks and then each strain was restreaked on CDM in the absence or presence of 12.5 mM butyrate and grown for 16 h at 37 °C in microaerobic conditions. Arylsulfatase assays were then performed. Each strain was tested in triplicate and each assay was performed three times. The level of expression of the transcriptional fusion in each strain was calculated relative to the expression in WT *C. jejuni* Δ*astA* strain grown in CDM in the absence of butyrate, which was set to 100 units.

Immunoblotting analysis of *C. jejuni* proteins
After growing *C. jejuni* strains from frozen stocks, strains were restreaked on MH, CDM alone, or CDM with 12.5 mM sodium butyrate with appropriate antibiotics and grown for 16 h at 37º C in microaerobic conditions. Cells were resuspended in PBS and diluted to OD_{600} 0.8. For whole cell lysates, 1 mL samples were centrifuged and washed once with 1 mL of PBS. Pellets were resuspended in 50 µl of 1x Laemmli buffer and 7.5 µl were loaded for detection of BumR and 10 µL for RpoA onto 10% SDS-PAGE gels. BumR M166 and RpoA M251 antisera were both used at 1:2000 dilution (5, 14) and incubated with membranes for 2 h. Secondary horseradish peroxidase (HRP)-conjugated goat antibody to detect each primary antibody was diluted 1:10,000 and incubated for 1 h. Immunoblots were developed by Western Lightning Plus ECL kit (Perkin-Elmer). To compare the levels of BumR in different WT *C. jejuni* and mutants, immunoblots were performed with three individual lysates for each strain. Densitometric analysis was performed using ImageJ to compare the levels of BumR to RpoA. All strains were compared to BumR:RpoA ratios of WT *C. jejuni*, which was set at 100.

Chick colonization assays. The ability of WT *C. jejuni* 81-176 *rpsL*^{Sm} and isogenic mutants to colonize chicks after oral inoculation was determined as previously described (15). Briefly, fertilized chicken eggs (SPAFAS) were incubated for 21 days at 37.5 °C with appropriate humidity and rotation in a Digital Sportsman model 1502 incubator (Georgia Quail Farms Manufacturing Company). One day after hatch, chicks were orally inoculated with 100 µL of phosphate buffered saline (PBS) containing approximately 10^2 CFU WT or mutant strains. Strains were prepared for infection after 16 h growth at 37 °C under microaerobic conditions on MH agar by suspending *C. jejuni* strains in MH broth. Dilution series in PBS were performed to achieve the appropriate inoculum for oral gavage of chicks. Dilutions of the inoculum were plated on MH agar to assess the number of bacteria in each inoculum. At 1, 4, 7, or 14 days post-infection, chicks were sacrificed, the proximal small intestine, distal small intestine, cecal, or large intestine contents were removed and suspended in PBS, and serial dilutions were plated on MH agar containing trimethoprim and cefoperazone. Following 72 h of growth at 37 °C in microaerobic conditions, bacteria were counted to determine CFU per gram of organ content. Recovered colonies were analyzed by colony PCR to verify that WT and mutant strains were isolated from respectively infected chicks.

Collection of RNA for RNAseq analysis. WT *C. jejuni* 81-176 *rpsL*^{Sm} (DRH212) and isogenic Δ*bumS* (PML360), and Δ*bumR* (PML335) mutants were grown from freezer stocks on MH agar containing 100 µg/mL streptomycin at 37 °C in microaerobic conditions for 48 h (5). Strains were then restreaked on MH agar containing trimethoprim and grown for an additional 16 h. *C. jejuni* growth was suspended from the plates in PBS and diluted into CDM with or without 12.5 mM butyrate to an OD_{600} of approximately 0.1. Strains were then grown statically at 37 °C in microaerobic conditions for 8 h to achieve mid-log phase growth. Following growth, strains were pelleted and resuspended in 2 mL RNA*later* Stabilization Solution (Invitrogen) and stored at 4 °C prior to RNA extraction.

Total RNA extraction was performed using PureLink RNA Mini Kit (Invitrogen). Depletion of rRNA, strand-specific library building, and sequencing was performed with Genewiz, Inc. Resultant reads were mapped to the published *C. jejuni* 81-176 genome using CLC Genomic Workbench Software (Qiagen). After local realignment, the reads per kilobase of transcript per million mapped reads (RPKM) metric was calculated for each annotated coding sequence, as described previously in (16, 17). Comparisons between genotypes were deemed significant if they had a greater than 3-fold change and an FDR p-value < 0.05.

Semiquantitative real-time RT-PCR (qRT-PCR) analysis. After growth of *C. jejuni* strains from freezer stocks on MH agar containing appropriate antibiotics, strains were restreaked on MH agar and grown for an additional 16 h. *C. jejuni* growth was then suspended from the plates in PBS and diluted into 25 mL of CDM alone or CDM with 100 mM potassium acetate, 12.5 mM sodium butyrate, or 25 mM sodium L-lactate (all buffered to pH 7.0). Strains were grown statically at 37 °C in microaerobic conditions for 8 h to achieve mid-log phase growth. Total RNA was extracted with RiboZol (Amresco) and RNA was treated with DNaseI (Invitrogen). RNA was diluted to a concentration of 5 ng/µL before analysis. qRT-PCR was performed using a 7500 real-time PCR system (Applied Biosystems) with *secD* or *recA* mRNA detection as an endogenous control. mRNA transcript levels in strains DRH212, DRH461, or KNG640 grown in CDM alone served as WT controls to determine relative gene expression in isogenic mutants.

Expression and purification of recombinant proteins

Expression of glutathione S-transferase (GST)-BumR recombinant proteins were purified as previously described with slight modifications (5). Briefly, expression plasmids were transformed into *E. coli* BL21 (DE3), grown in 2xYT medium, and induced with 300 µM IPTG. GST-BumR proteins were purified from the soluble fraction with glutathione Sepharose beads (GE Healthcare). Following GST tag cleavage by thrombin and removal of thrombin by addition of benzamidine Sepharose following manufacturer's instructions (GE Healthcare), the recombinant protein was recovered. Glycerol was added to a final concentration of 10% and proteins were stored at -80°C. Expression of FlgR constructs for purification of proteins with C-terminal 6XHis-tags was performed as previously described (18, 19). Expression of FlgS with an N-terminal 6XHis-tag was performed as previously described (20).

Primers containing in-frame 5' BamHI restriction sites to codon 2 and the stop codon were used to amplify WT *bumS* from *C. jejuni* 81-176 genomic DNA. Following restriction digestion, the fragments were ligated into the BamHI site of pGEX-4T-2 to generate pPML204. Primers were also designed to amplify *bumS* to encode mutations in the H box (QW196-7EL), in the D box (A338G), and in both the H box and D box (QW196-7EL with A338G). These fragments were inserted into BamHI-digested pGEX-4T-2 by Gibson Assembly to create to pKNG610 (pGEX-4T-2::$bumS_{QW196\text{-}7EL}$), pKNG611 (pGEX-4T-2::$bumS_{A338G}$), and the triple mutant pKNG612 (pGEX-4T-2::$bumS_{HD\ box}$).

For protein expression, plasmids were transformed into *E. coli* BL21 (DE3) and plated on LB supplemented with 100 µg/mL ampicillin and 0.5% D-(+)-Glucose. After overnight growth, single colonies were inoculated into 10 mL of 2xYT media supplemented with 100 µg/mL ampicillin and 1% D-(+)-Glucose and grown until OD_{600} of 0.6-0.8. Cultures were then diluted 1:100 into 500 mL of 2xYT supplemented with 100 µg/mL ampicillin and 1% D-(+)-Glucose and grown until OD_{600} of 0.8. Cultures were then induced with 50 µM IPTG overnight at 16 °C. Recombinant protein was purified from the soluble fraction with glutathione Sepharose beads (GE Healthcare) in a lysis buffer containing 50 mM Tris-HCL pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT. Protein was eluted in lysis buffer containing 20 mM glutathione and dialyzed into lysis buffer supplemented with 10% glycerol. Proteins were stored at -80 °C.

***In vitro* kinase assays with ATP[γ-^{32}P].**
In vitro kinase assays were performed as previously described with minor modifications (21). Recombinant WT BumS or BumS mutant proteins with N-terminal GST-fusions or 6XHis-FlgS (10 pmol) were incubated in buffer containing 10 mM HEPES pH 8.0, 50 mM potassium chloride, 10 mM magnesium chloride, 2 mM DTT, 0.1 mM EDTA, 0.5 mM ATP, 10 µCi ATP[γ-^{32}P], and 10% glycerol in 15 µL total volume for indicated time at 37 °C. Reactions were terminated with 2X Laemmli buffer, and proteins were separated by 12.5% SDS-PAGE without boiling. Gels were dried and then analyzed with a Typhoon FLA 9500 phosphorimager according to manufacturer's instructions (Amersham Biosciences).

***In vitro* phosphatase assays with Ac[^{32}P] autophosphorylation.**
Ac[^{32}P] was generated as previously described with some modifications (19). For BumR phosphorylation, 0.3 units of *E. coli* acetate kinase (AckA; Sigma-Aldrich) were incubated in buffer containing 50 mM Tris HCl pH 7.6, 120 mM potassium acetate, 20 mM magnesium chloride, 1 mM DTT, 0.5 mM ATP, 5 µCi ATP[γ-^{32}P], and 10% glycerol in 7.5 µL total volume for 2 h at room temperature. BumR (20 pmol) was then added to the reaction to 10 µL total volume for 20 min at 37 °C. 6XHis-FlgS or WT BumS or BumS mutants with N-terminal GST-fusions were then added to achieve a 1:10 to 1:1 BumS:BumR or 1:2 FlgS:BumR ratio, respectively, in 20 µL total volume and incubated for 1 to 60 minutes at 37 °C. In some assays, butyrate was also added up to 12.5 mM. For $FlgR_{\Delta CTD}$ phosphorylation, 0.5 units of AckA were incubated in buffer containing 50 mM Tris HCl pH 7.6, 120 mM potassium acetate, 20 mM magnesium chloride, 1 mM DTT, 0.5 mM ATP, 25 µCi ATP[γ-^{32}P], and 10% glycerol in 7.5 µL total volume for 2 h at room temperature. $FlgR_{\Delta CTD}$ (50 pmol) was then added to the reaction to 12 µL total volume for 20 min at 37 °C. GST-BumS was then added to achieve a 1:2 or 1:1 BumS: $FlgR_{\Delta CTD}$ ratio in 20 µL total volume and incubated for 10 min at 37 °C. Reactions were terminated with 2X Laemmli buffer, and proteins were separated by 12.5% SDS-PAGE without boiling.

To directly compare BumR and $FlgR_{\Delta CTD}$ autophosphorylation, 0.5 units of AckA were incubated in buffer containing 50 mM Tris HCl pH 7.6, 120 mM potassium acetate, 20 mM magnesium chloride, 1 mM DTT, 0.5 mM ATP, 25 µCi ATP[γ-^{32}P], and 10% glycerol in 7.5 µL total volume for 2 h at room temperature. BumR or $FlgR_{\Delta CTD}$ (50 pmol) was then added to the reaction to 12.5 µL total volume for 20 min at 37 °C. Reactions were terminated with 2X Laemmli buffer, and BumR (diluted 1:1000 to 50 fmol) and $FlgR_{\Delta CTD}$ (50 pmol) autophosphorylation reactions were separated by 12.5% SDS-PAGE without boiling. Gels were analyzed as described above.

Electrophoretic mobility shift assays (EMSAs). BumR was purified as described above. Promoter DNA fragments relative to the translational start site were amplified as follows: -286 to +35 for the *peb3*, -448 to +28 for *Cjj0580*, -453 to +46 for *cetA*, and -299 to +29 for *recA* promoters. EMSAs were performed based on a modified protocol (22). Briefly, 0 to 1.5 µM of WT BumR in the presence or absence of 50 mM lithium acetyl-phosphate (Li-AcP) was

incubated with ^{32}P-labelled DNA at 42 °C for 20 min as indicated. Competition experiments were performed through the addition of unlabeled DNA for the genes mentioned above and *recA* as a non-specific control. Unlabeled DNA was added at increasing ratios relative to ^{32}P-labeled DNA, and 1.5 μM of BumR pretreated with Li-AcP was used. After electrophoresis and drying of gels, analysis was performed with a Typhoon FLA 9500 phosphorimager according to manufacturer's instructions (Amersham Biosciences).

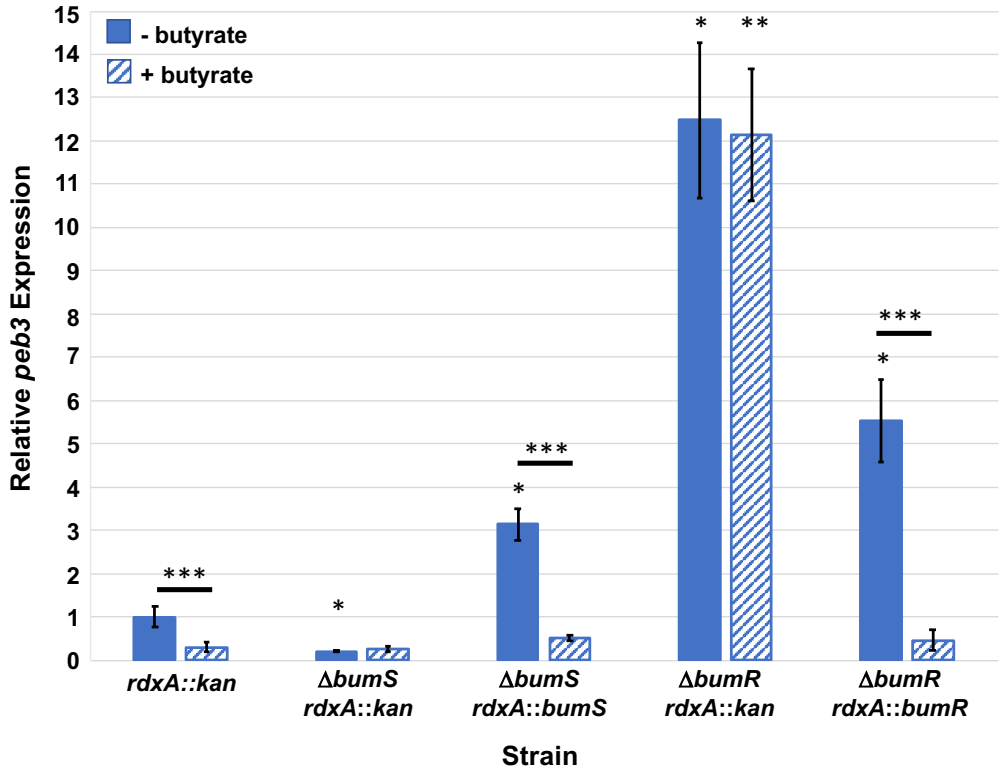

Figure S1. Complementation of *C. jejuni* Δ*bumS* and Δ*bumR* mutants. Semi-quantitative real-time PCR (qRT-PCR) analysis of *peb3* transcription in *C. jejuni rdxA*::*kan* (which serves as the WT control) and Δ*bumS* or Δ*bumR* mutants with *in cis* complementation of the respective gene with the native promoter inserted into *rdxA*. Uncomplemented Δ*bumS* or Δ*bumR* mutants only contained a kanamycin-resistance cassette in *rdxA*. All strains were grown in CDM alone or CDM supplemented with 12.5 mM butyrate. The expression of *peb3* in CDM alone (solid blue bars) and CDM with butyrate (hatched blue bars) in *C. jejuni rdxA*::*kan* and mutant strains are shown. The level of *peb3* transcription in *C. jejuni rdxA*::*kan* grown in CDM alone as measured by qRT-PCR was set to 1. Expression of *peb3* in *C. jejuni rdxA*::*kan* grown in CDM with butyrate or mutants grown with or without butyrate is shown relative to *C. jejuni rdxA*::*kan* grown in CDM alone. Results from a representative assay with each sample analyzed in triplicate are shown. Error bars indicate standard deviations of the average level of expression from three samples. Statistical significance of ΔC_T values relative to *secD* reference gene was calculated in GraphPad Prism by ANOVA with Tukey's test: *, indicates the mutant grown in CDM alone had significantly increased or decreased expression relative to *C. jejuni rdxA*::*kan* grown in CDM alone; **, indicates the mutant grown with butyrate had a significantly increased or decreased expression relative to *C. jejuni rdxA*::*kan* grown with butyrate; ***, indicates a strain showed a significantly different level of expression when grown in the presence of butyrate compared to growth in CDM alone ($P < 0.05$).

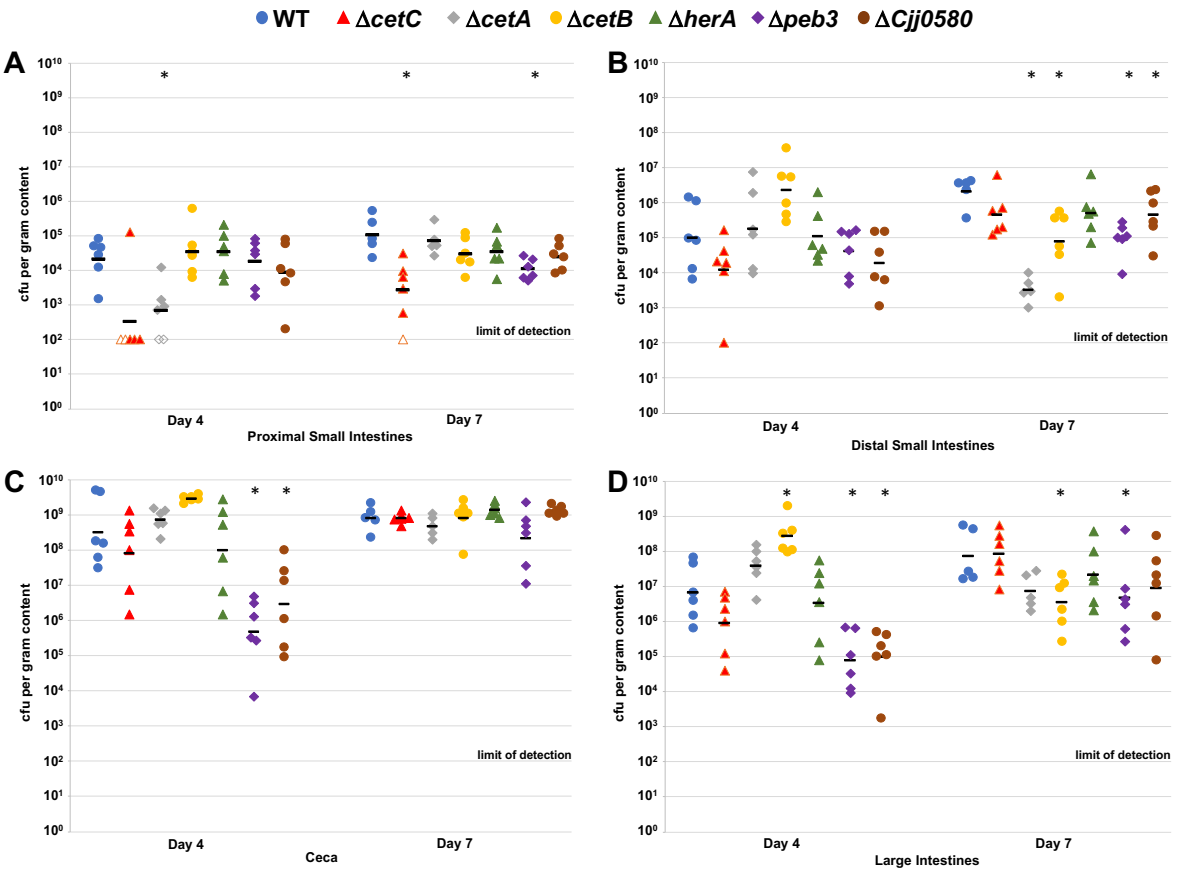

Figure S2. Colonization dynamics of WT *C. jejuni* and isogenic mutants lacking a gene within the BumSR regulon. Day of hatch chicks were orally infected with approximately 100 CFU of WT *C. jejuni* 81-176 *rpsL*^{Sm} (blue circles), isogenic Δ*cetC* (red triangles), Δ*cetA* (grey diamonds), Δ*cetB* (yellow circles), Δ*herA* (green triangles), Δ*peb3* (purple diamonds), and Δ*Cjj0580* (brown circles) mutants. Chicks were sacrificed at day 4 or 7 post-infection and the levels of each *C. jejuni* strain in (A) the proximal small intestine, (B) distal small intestine, (C) ceca, and (D) large intestine was determined (reported as CFU per gram of content). Each closed circle represents the level of *C. jejuni* in a single chick. Open circles represent chicks with *C. jejuni* levels below the limit of detection (<100 CFU per gram of content). Horizontal bars represent geometric mean for each group. Statistical analysis was performed using the Mann-Whitney *U* test (* $P < 0.05$).

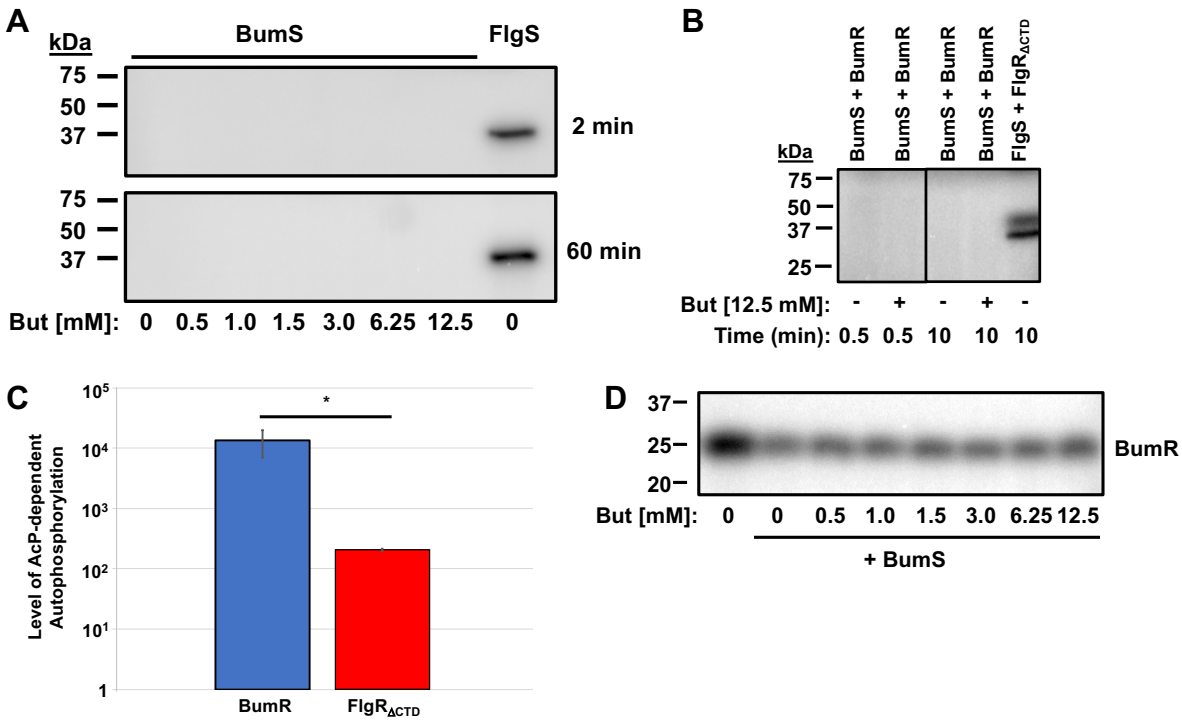

Figure S3. BumS kinase and phosphatase activity in the presence of butyrate.
(A) Representative autokinase assay of WT BumS alone or in the presence of increasing amounts of butyrate. Autokinase assays were performed with WT BumS and [γ-^{32}P]ATP. FlgS was included as a positive control. (B) Representative autokinase and phosphotransfer assay of WT BumS in the presence of BumR and butyrate. Recombinant BumS and BumR were mixed together at a 1:2 BumS:BumR ratio with [γ-^{32}P]ATP and without or with 12.5 mM butyrate for indicated times. As a control, FlgS and FlgR$_{\Delta CTD}$ were mixed at a 1:2 FlgS:FlgR$_{\Delta CTD}$ ratio with [γ-^{32}P]ATP for indicated time. (C) Quantitation of *in vitro* autophosphorylation of BumR and FlgR$_{\Delta CTD}$ using Ac[^{32}P] as a phosphodonor. After incubation of BumR and FlgR$_{\Delta CTD}$ with Ac[^{32}P], ^{32}P-labelled protein was quantitated by phosphorimaging of acrylamide gels to determine relative signal per pmol of protein. Statistical analysis was performed using the Student *t*-test of log transformed data to compare the relative autophosphorylation of BumR to FlgR$_{\Delta CTD}$ (* $P <$ 0.05). (D) Representative assay of phospho-BumR dephosphorylation by BumS with increasing concentrations of butyrate. BumR was autophosphorylated with *in vitro* generated Ac[^{32}P] prior to addition of BumS at a 1:2 BumS:BumR ratio and butyrate at different concentrations.

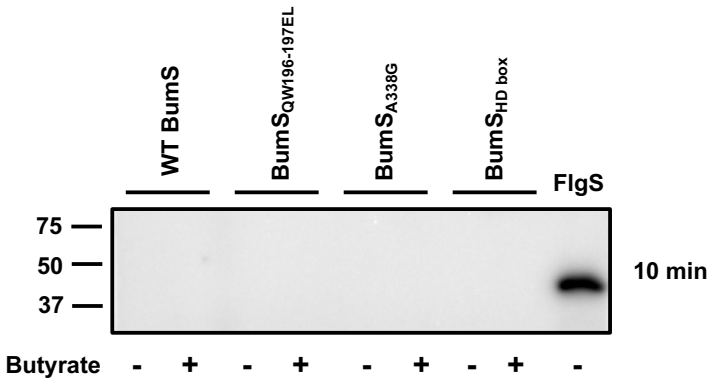

Figure S4. Autokinase activity of WT BumS and BumS H and D box mutants. WT BumS, BumS$_{QW196-7EL}$ (H box mutant), BumS$_{A338G}$ (D box mutant), and BumS$_{HD box}$ (that combines the QW196-7EL and A338G mutations) were incubated with [γ-^{32}P]ATP. WT FlgS was included as a positive control for autokinase activity of a *C. jejuni* histidine sensor kinase.

Table S1. Condensed list of genes differentially expressed in WT *C. jejuni* 81-176 grown with or without butyrate analyzed by RNAseq[1]

		WT+butyrate/WT	
Gene[2]	**Predicted/Known Function of Encoded Protein**	**Fold-change**	**FDR p-value**
Cjj81176_0315	Peb3 glycoprotein for transport of phosphate-containing metabolites	-7.56	0
Cjj81176_1657	DUF411 domain-containing hypothetical protein	-3.96	0
Cjj81176_0211	Fe(3+) ABC transporter substrate-binding protein	-3.48	0
Cjj81176_1656	Thioredoxin family protein	-2.57	1.61E-03
Cjj81176_0580	Putative di- and tri-carboxylate transporter	-2.22	9.02E-06
Cjj81176_1204	CetB methyl-accepting chemotaxis protein	-2.19	1.88E-03
Cjj81176_1628	Putative TPR-domain and zinc-finger containing protein	12.22	1.29E-04
Cjj81176_1623	Putative periplasmic protein	9.17	0.05
Cjj81176_0439	Putative oxidoreductase	4.70	0
Cjj81176_0438	Gluconate 2-dehydrogenase subunit family 3	4.59	0
Cjj81176_0885	Putative cytochrome c protein	3.03	0

[1] WT *C. jejuni* 81-176 *rpsL*^{Sm} was grown in CDM alone or CDM with 12.5 mM butyrate at 37 °C in microaerobic conditions for 8 h.

[2] Select genes encoding proteins with at least two-fold changes in transcription after growth in the presence or absence of butyrate are indicated. The effects of butyrate on transcription of each *C. jejuni* gene is displayed in Dataset S1.

Table S2. Condensed list of genes differentially expressed in WT *C. jejuni* 81-176 and isogenic Δ*bumS* or Δ*bumR* mutants analyzed by RNAseq[1]

		Δ*bumS* / WT *C. jejuni*[3]		Δ*bumR* / WT *C. jejuni*[3]	
Gene[2]	**Predicted/Known Function of Encoded Protein**	**Fold change**	**FDR p-value**	**Fold change**	**FDR p-value**
Cjj81176_1326	Putative acyl carrier protein	-6.04	1.04E-03	-10.20	4.86E-07
Cjj81176_0266	HerA hemerythrin	-5.85	3.09E-05	-4.55	2.86E-05
Cjj81176_0315	Peb3 glycoprotein for transport of phosphate-containing metabolites	-4.90	0	5.28	0
Cjj81176_1206	CetC methyl-accepting chemotaxis protein	-4.70	7.77E-04	-5.66	2.99E-07
Cjj81176_1512	DUF4435 domain-containing hypothetical protein	-3.53	6.59E-04	-3.97	2.58E-07
Cjj81176_1666	CgpD AMIN domain-containing glycoprotein	-3.20	8.82E-06		
Cjj81176_0195	Putative 6-pyruvoyl tetrahydrobiopterin synthase	-3.10	2.55E-06	-3.94	4.65E-07
Cjj81176_0967	LolA lipoprotein chaperone	-3.07	0.02	-4.58	4.86E-06
Cjj81176_0267	Hypothetical protein	-3.01	4.94E-03	-5.89	4.71E-09
Cjj81176_0438	Gluconate 2-dehydrogenase subunit family 3	6.82	0	3.06	0
Cjj81176_0439	Putative oxidoreductase	6.59	0		
Cjj81176_0885	Putative cytochrome c protein	4.20	0		
Cjj81176_1485	Sulfite exporter TauE/SafE family protein	3.95	1.22E-04		
Cjj81176_1097	Hypothetical protein			-6.10	4.28E-03
Cjj81176_1476	Putative lipoprotein			-5.81	0
Cjj81176_1154	CgtA β-1,4-N-acetylgalactosaminyltransferase			-5.75	0
	RpsU ribosomal protein S21			-5.25	0
Cjj81176_1257	Hypothetical protein			-4.85	0
Cjj81176_1171	Cytochrome oxidase maturation protein			-4.69	1.00E-04
Cjj81176_0846	Predicted CoA-binding protein			-4.61	4.55E-05
Cjj81176_0985	GNAT family acetyltransferase			-4.00	2.70E-04
Cjj81176_1314	Predicted class I SAM-dependent methyltransferase			-3.91	1.15E-09
Cjj81176_0705	Hypothetical protein			-3.86	2.56E-11
Cjj81176_0075	Cytochrome c family protein			-3.72	0
Cjj81176_0484	Hypothetical protein			-3.71	0
Cjj81176_0010	Lipooligosaccharide core biosynthesis protein			-3.49	6.53E-03
Cjj81176_1208	Hypothetical protein			-3.41	0

Cjj81176_1434	Putative sugar transferase	-3.40	1.61E-13
Cjj81176_0400	Hypothetical protein	-3.40	8.27E-04
Cjj81176_1431	Putative sugar transferase	-3.28	1.11E-08
Cjj81176_0117	DUF4492 domain-containing protein	-3.26	0.05
Cjj81176_1358	Hypothetical protein	-3.19	4.81E-13
Cjj81176_1204	CetB methyl-accepting chemotaxis protein	-3.19	0
Cjj81176_0421	tRNA amidotransferase	-3.18	2.35E-11
Cjj81176_0196	Radical SAM domain-containing protein	-3.17	5.24E-05
Cjj81176_1732	Hypothetical protein	-3.05	2.54E-09
Cjj81176_1238	Putative DNA-binding protein	-3.05	7.78E-05
Cjj81176_0192	16S rRNA methyltransferse	-3.02	3.83E-04
Cjj81176_0064	Predicted cytochrome c family protein	5.12	0
Cjj81176_1006	Possible phase-variable major facilitator superfamily protein	4.83	2.21E-08
Cjj81176_0063	Predicted cytochrome c protein	4.59	0
Cjj81176_1619	ExbB TonB-system transport protein	4.37	0
Cjj81176_0372	Tryptophan synthase beta chain subunit	3.58	3.48E-07
Cjj81176_0580	Putative di- and tri-carboxylate transporter	3.39	0
Cjj81176_1526	PxpA 5-oxoprolinase subunit A	3.30	5.28E-07
Cjj81176_0211	Fe(3+) ABC transporter substrate-binding protein	3.04	4.73E-06

[1] WT *C. jejuni* 81-176 *rpsL*^{Sm} and isogenic Δ*bumS* and Δ*bumR* mutants were grown in CDM alone at 37 °C in microaerobic conditions for 8 h.
[2] Select genes encoding proteins with at least three-fold changes in transcription between WT and mutant strains. The effects of *bumS* or *bumR* deletion on transcription of each *C. jejuni* gene is displayed in Datasets S2 and S3.
[3] The lack of a value for a transcriptional difference for a gene reported for both Δ*bumS* and Δ*bumR* relative to WT indicates that the gene only had a significant transcriptional alteration in only Δ*bumS* or Δ*bumR* as revealed by RNAseq analysis.

Table S3. Bacterial strains used in this study

Strain	Genotype	Source/Reference
DH5α	E. coli *supE44* Δ*lacU169* (ϕ80*lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Invitrogen
BL21 (DE3)	*E. coli fhuA2 [lon] ompTgal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*	New England Biolabs
BL21 (DE3)/pLysE	*E. coli* F- *ompT hsdS*$_B$(r_B- m_B-) *gal dcm* (DE3) pLysE Cm^R	Sigma-Aldrich
XL1-Blue	*E. coli recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F´ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^R)]	Agilent
DRH212	81–176 *rpsL*^{Sm}	(2)
DRH307	81–176 *rpsL*^{Sm} Δ*cetB*	(2)
DRH333	81–176 *rpsL*^{Sm} Δ*cetA*	(2)
DRH461	81–176 *rpsL*^{Sm} Δ*astA*	(4)
DRH1159	81–176 *rpsL*^{Sm} *cetC*::*cat-rpsL*	(15)
KNG248	81–176 *rpsL*^{Sm} Δ*peb3*	This study
KNG467	81–176 *rpsL*^{Sm} Δ*astA bumR*$_{\Delta KFK}$	This study
KNG558	81–176 *rpsL*^{Sm} *Cjj0580*::*cat-rpsL*	This study
KNG640	81-176 *rpsL*^{Sm} Δ*astA rdxA*::*kan*	This study
KNG643	81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS rdxA*::*kan*	This study
KNG646	81-176 *rpsL*^{Sm} Δ*astA* Δ*bumS rdxA*::*bumS-kan*	This study
KNG649	81-176 *rpsL*^{Sm} Δ*astA* Δ*bumR rdxA*::*kan*	This study
KNG652	81-176 *rpsL*^{Sm} Δ*astA* Δ*bumR rdxA*::*bumR-kan*	This study
PML318	81-176 *rpsL*^{Sm} Δ*astA bumS*::*cat-rpsL*	This study
PML324	81-176 *rpsL*^{Sm} Δ*astA bumR*::*cat-rpsL*	(5)
PML335	81–176 *rpsL*^{Sm} Δ*bumR*	(5)
PML337	81–176 *rpsL*^{Sm} Δ*astA* Δ*bumR*	(5)

PML360	81–176 *rpsL*^{Sm} Δ*bumS*	(5)
PML363	81–176 *rpsL*^{Sm} Δ*astA* Δ*bumS*	This Study
PML739	81–176 *rpsL*^{Sm} Δ*astA bumR*$_{D58E}$	(5)
PML769	81–176 *rpsL*^{Sm} Δ*astA bumR*$_{D58N}$	(5)
PML908	81–176 *rpsL*^{Sm} Δ*astA* Δ*bumR peb3*::*astA-kan*	This study
PML911	81–176 *rpsL*^{Sm} Δ*astA* Δ*bumS peb3*::*astA-kan*	This study
PML921	81–176 *rpsL*^{Sm} Δ*astA peb3*::*astA-kan*	This study
ABT749	81–176 *rpsL*^{Sm} *herA*::*cat-rpsL*	(6)
ABT754	81–176 *rpsL*^{Sm} Δ*herA*	This study
WPK742	81–176 *rpsL*^{Sm} *peb3*::*cat-rpsL*	This study

Table S4. Plasmids used in this study

Plasmid	Genotype	Source/Reference
pUC19	Amp^R	New England Biolabs
pGEX-4T-2	Amp^R	GE Healthcare
pABT745	pUC19 with an in-frame fusion of the stop and start codons of *herA* with approximately 750 bases of upstream and downstream DNA cloned into the BamHI site	(6)
pABT1307	pUC19::*rdxA*::*kan*	(7)
pDRH265	pUC19::*cat-rpsL*	(2)
pDRH580	pUC19::*astA-kan*	(4)
pDRH1336	pQE30::*flgS*	(20)
pDRH2445	pSpaceball1 containing the *darkhelmet Tn*	(9)
pKNG210	pUC19 with an in-frame fusion of start and stop codon of *peb3* with approximately 500 bases of upstream and downstream DNA cloned into PstI site	This study
pKNG327	pUC19 with an in-frame deletion of 9 bases removing codons for a KFK unit within the KFKKFK motif at position 45-51 of BumR with approximately 600 bases of upstream and downstream DNA cloned into PstI site	This study
pKNG537	pUC19 with DNA containing the *Cjj81176_0580* locus with approximately 750 bases upstream and downstream sequence that also includes a A777C mutation to result in an EcoRV site within the gene cloned into PstI site	This study
pKNG544	pKNG537 with a *cat-rpsL* inserted into the EcoRV site of *Cjj81176_0580*	This study
pKNG610	pGEX-4T-2 with $bumS_{QW196\text{-}7EL}$ coding sequence from codon 2 to the stop codon cloned into the BamHI site	This study
pKNG611	pGEX-4T-2 with $bumS_{A338G}$ coding sequence from codon 2 to the stop codon cloned into the BamHI site	This study
pKNG612	pGEX-4T-2 with $bumS_{HD\ box}$ coding sequence from codon 2 to the stop codon cloned into the BamHI site	This study
pKNG636	pABT1307 with DNA from approximately 300 bases upstream of *bumS* through the coding sequence cloned into BglII site in *rdxA*	This study
pKNG637	pABT1307 with DNA amplified from PML363 with approximately 300 bases upstream of *bumS* through the end of *bumR* coding sequence cloned into BglII site in *rdxA*	This study
pPML101	pUC19 with *bumSR* locus cloned into the BamHI site with a mutation to generate a HpaI site in *bumS*	(5)
pPML106	pPML101 with *cat-rpsL* cloned into the HpaI site in *bumS*	This study
pPML165	pGEX-4T-2 with *bumR* coding sequence from codon 2 to the stop codon cloned into BamHI site	(5)
pPML204	pGEX-4T-2 with *bumS* coding sequence from codon 2 to the stop codon cloned into the BamHI site	This study
pPML334	pUC19::Δ*bumS*	(5)
pPML849	pUC19 with *peb3* locus from 81-176 cloned into the BamHI site	This study
pPML873	pPML849 with an *astA-kan* inserted into the BsaBI site of *peb3*	This study

pSNJ753	pT7-7::*flgR*$_{\Delta CTD}$-*6XHis*	(18)
pWPK740	pPML849 with a *cat-rpsL* inserted into BsaBI site of *peb3*	This study

Dataset S1. Differential gene expression in WT *C. jejuni* grown in CDM with 12.5 mM butyrate relative to WT C. jejuni grown in CDM alone

WT *C. jejuni* 81-176 *rpsL*^{Sm} was grown in CDM alone or CDM with 12.5 mM butyrate at 37 °C in microaerobic conditions for 8 h. Three biological replicates were examined for each condition. A three-fold change in gene expression with a false discovery rate (FDR) corrected p value of < 0.05 were considered as significant differences WT strains grown in the two conditions. A FDR p value of zero represents a p value of < 1E-14.

Dataset S2. Differential gene expression in *C. jejuni* Δ*bumS* grown in CDM alone relative to WT *C. jejuni* grown in CDM alone.

WT *C. jejuni* 81-176 *rpsL*^{Sm} and the isogenic Δ*bumS* mutant were grown in CDM without butyrate at 37 °C in microaerobic conditions for 8 h. Three biological replicates were examined for each strain. A three-fold change in gene expression with a false discovery rate (FDR) corrected p value of < 0.05 were considered as a significant differences between WT *C. jejuni* and the Δ*bumS* mutant. A FDR p value of zero represents a p value of < 1E-14.

Dataset S3. Differential gene expression in *C. jejuni* Δ*bumR* grown in CDM alone relative to WT *C. jejuni* grown in CDM alone

WT *C. jejuni* 81-176 *rpsL*^{Sm} and the isogenic Δ*bumR* mutant were grown in CDM without butyrate at 37 °C in microaerobic conditions for 8 h. Three biological replicates were examined for each strain. A three-fold change in gene expression with a false discovery rate (FDR) corrected p value of < 0.05 were considered as a significant differences between WT *C. jejuni* and the Δ*bumR* mutant. A FDR p value of zero represents a p value of < 1E-14.

Dataset S4. Differential gene expression in *C. jejuni* Δ*bumS* grown in CDM with 12.5 mM butyrate relative to WT *C. jejuni* grown in CDM with 12.5 mM butyrate

WT *C. jejuni* 81-176 *rpsL*^{Sm} and the isogenic Δ*bumS* mutant were grown in CDM with 12.5 mM butyrate at 37 °C in microaerobic conditions for 8 h. Three biological replicates were examined for each strain. A three-fold change in gene expression with a false discovery rate (FDR) corrected p value of < 0.05 were considered as a significant differences between WT *C. jejuni* and the Δ*bumS* mutant. A FDR p value of zero represents a p value of < 1E-14.

Dataset S5. Differential gene expression in *C. jejuni* Δ*bumR* grown in CDM with 12.5 mM butyrate relative to WT *C. jejuni* grown in CDM with 12.5 mM butyrate

WT *C. jejuni* 81-176 *rpsL*^{Sm} and the isogenic Δ*bumR* mutant were grown in CDM with 12.5 mM butyrate at 37 °C in microaerobic conditions for 8 h. Three biological replicates were examined for each strain. A three-fold change in gene expression with a false discovery rate (FDR) corrected p value of < 0.05 were considered as a significant differences between WT *C. jejuni* and the Δ*bumR* mutant. A FDR p value of zero represents a p value of < 1E-14.

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