Novel Components of the Flagellar System in Epsilonproteobacteria

Belle Gao,a* Maria Lara-Tejero,a* Matthew Lefebre,a Andrew L. Goodman,a,b Jorge E. Galán*a

Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut, USAa; Microbial Diversity Institute, Yale University School of Medicine, New Haven, Connecticut, USAa

ABSTRACT Motility is essential for the pathogenesis of many bacterial species. Most bacteria move using flagella, which are multiprotein filaments that rotate propelled by a cell wall-anchored motor using chemical energy. Although some components of the flagellar apparatus are common to many bacterial species, recent studies have shown significant differences in the flagellar structures of different bacterial species. The molecular bases for these differences, however, are not understood. The flagella from epsilonproteobacteria, which include the bacterial pathogens Campylobacter jejuni and Helicobacter pylori, are among the most divergent. Using next-generation sequencing combined with transposon mutagenesis, we have conducted a comprehensive high-throughput genetic screen in Campylobacter jejuni, which identified several novel components of its flagellar system. Biochemical analyses detected interactions between the identified proteins and known components of the flagellar machinery, and in vivo imaging located them to the bacterial poles, where flagella assemble. Most of the identified new components are conserved within but restricted to epsilonproteobacteria. These studies provide insight into the divergent flagella of this group of bacteria and highlight the complexity of this remarkable structure, which has adapted to carry out its conserved functions in the context of widely diverse bacterial species.

IMPORTANCE Motility is essential for the normal physiology and pathogenesis of many bacterial species. Most bacteria move using flagella, which are multiprotein filaments that rotate propelled by a motor that uses chemical energy as fuel. Although some components of the flagellar apparatus are common to many bacterial species, recent studies have shown significant divergence in the flagellar structures across bacterial species. However, the molecular bases for these differences are not understood. The flagella from epsilonproteobacteria, which include the bacterial pathogens Campylobacter jejuni and Helicobacter pylori, are among the most divergent. We conducted a comprehensive genetic screen in Campylobacter jejuni and identified several novel components of the flagellar system. These studies provide important information to understand how flagella have adapted to function in the context of widely diverse sets of bacterial species and bring unique insight into the evolution and function of this remarkable bacterial organelle.

The bacterial flagellum is a very complex nanomachine that is highly conserved across bacterial species (1–3). Despite this conservation, there is significant variation in the numbers and the locations of flagella on the bacterial body (4). While some bacteria have multiple peritrichous flagella, others have a single flagellum at one (unipolar or monotrichous) or both (bipolar or amphitrichous) poles or have flagella embedded within the periplasmic space (5–8). Numerous studies, mostly carried out in the model organisms Salmonella enterica serovar Typhimurium and Escherichia coli, have provided detailed information about the composition, structure, assembly, and function of this remarkable organelle (3, 9). These studies have shown that the bacterial flagellum consists of two main structural components, the hook basal body complex and the extracellular filament. In addition, a number of nonstructural components are required for flagellar assembly and function. The deployment of the flagellar apparatus is highly regulated by a complex regulatory network that ensures the expression of its components at the appropriate time and within the appropriate environment (3). Although the general organization of flagella is highly conserved, it has recently become apparent that there is considerable diversity in the flagellar structure itself across bacterial species (1, 10, 11). In particular, cryo-electron tomography studies have shown that flagella from members of the Epsilonproteobacteria are among the most structurally diverse, exhibiting several unique structural features that most likely correspond to novel, yet unidentified, flagellar components (12). Understanding the molecular bases for these differences would be important to understand the evolution and adaptation of this bacterial organelle across bacterial species.

All members of the Epsilonproteobacteria have their flagella located at either one or both cellular poles (13–19). Campylobacter jejuni and Helicobacter pylori are the most studied epsilonproteobacteria because they are important human pathogens. C. jejuni is a major cause of food-borne illness (20, 21), while H. pylori is an important cause of stomach ulcers and gastric cancer (22, 23). Motility is essential for these bacteria to invade cultured cells,
colocalize animals, and cause disease in susceptible hosts (24–27). In addition to their unique structural features revealed by cryo-electron tomography (12), Campylobacter and Helicobacter flagella exhibit unique aspects in the regulation of the expression of their flagellar genes and in the assembly of their flagellar structure (28, 29). For example, C. jejuni flagellar assembly, which occurs at the poles, requires the posttranslational glycosylation of the flagellin subunits and is specifically coordinated with cell division (30–33). Regulation of flagellar gene expression in Campylobacter and Helicobacter is also unique, involving a two-component system (FlgRS), the FlhF GTPase, and the transcription factors sigma54 and sigma28 (34–36). Here we have identified several novel components of the C. jejuni flagellar system through a high-throughput screen. We present data that collectively indicate that these novel genes encode factors that directly influence motility. The newly identified components are highly conserved in but largely restricted to Epsilonproteobacteria. This report significantly enhances our understanding of the unique flagellar system of these bacterial taxa and highlights the versatility of this nanomachine with respect to its ability to adapt its design to the specific needs of diverse bacteria.

RESULTS

Construction and characterization of a C. jejuni transposon mutant library by INSeq insights into essential genes. Transposon mutagenesis coupled to next-generation sequencing is a powerful tool to carry out high-throughput mutant screens in bacteria (37–39). We established a genome-wide disruption library in C. jejuni 81-176 with a sequencing-adapted mariner transposon derivative specifically tailored for its use in this bacterium. The transposable element has two antibiotic resistance markers flanked by modified mariner inverted repeats containing an Mmel restriction site (Fig. 1A). The two resistance genes lack transcription terminators and are arranged in opposite orientations so that their own promoters point outward of the transposable element, thus minimizing polar transcription effects on downstream genes. The transposon mutant library was constructed after in vitro transposition and natural transformation as previously described (40). Characterization of the mutant library by nucleotide sequencing of the transposon insertion sites indicated that the library consists of ~50,000 transposon insertion mutants with insertions well distributed across the genome at an average density of 31 insertions/kb (Fig. 1B; see also Table S1 in the supplemental material). To validate the reproducibility of high-throughput insertion sequencing (INSeq), we performed technical and biological replicates, which indicated a very high degree of reproducibility (Fig. 1C).

After filtering out insertions within the last 20% of any coding region (because such insertions could permit gene function), we found that 1,583 of the 1,758 predicted open reading frames (ORFs) in the C. jejuni genome and its two resident plasmids (pTet and pVir) had been directly disrupted in the mutant pool. Insertions were obtained for all but 175 predicted open reading frames, making these genes potential candidates to be identified as essential under the growth conditions used in this study (Table S2). This set of genes, however, shows little overlap with two recently reported lists of potential essential genes for the C. jejuni NCTC11168 strain (41, 42). Besides differences in the strains, there are significant experimental differences between the previous studies and those described here which could account for the discrepancies. The previous studies, which identified 195 and 233 potential essential genes, involved a much smaller transposon insertion library (~7,000 insertions) and used microarrays instead of next-generation sequencing to determine the transposon insertion sites. The use of microarrays does not allow the elimination of insertions close to the 3′ end of an open reading frame, which may lead to insertions resulting in a functional product. Furthermore, the transposon element used in our study minimized polar transcriptional effects, potentially allowing insertions upstream of a cistron to be masked.

Identification of C. jejuni motility genes by searching for mutants unable to invade cultured mammalian cells. Key structural features of the flagellar apparatus in Epsilonproteobacteria diverge from those of model organisms such as E. coli and S. Typhimurium (12). These differences are more than likely attributable to unidentified flagellar components specific to Epsilonproteobacteria. These components cannot be identified with strategies that rely on amino acid sequence homology to known flagellar proteins (43, 44). Consequently, we set out to carry out a genetic screen to identify flagellar components unique to Campylobacter and other Epsilonproteobacteria. Previous genetic screens for C. jejuni nonmotile mutants have relied on assays that are not amenable to high throughput and therefore have not been comprehensive (40, 45, 46). Here, we carried out a high-throughput genetic screen that comprehensively searched the virulent strain of C. jejuni 81-176 for nonmotile mutants. We took advantage of the observation that motility is strictly required for the ability of C. jejuni to invade cultured mammalian cells (26, 47) and that the examination of the cultured-cell invasion phenotype is amenable to high-throughput screening. We therefore used INSeq to screen our comprehensive transposon insertion library (see above) for mutants unable to invade cultured mammalian cells (see details in Materials and Methods) to compare the representation of insertion mutants in the bacterial inoculum with that in the bacteria obtained after the invasion assay (37, 48).

Our screen identified mutants with mutations in 36 genes with drastic defects in their ability to invade cultured mammalian cells (Table 1 and Fig. 1D; see also Table S1 in the supplemental material), which is the expected phenotype of nonmotile mutants. Four of the genes had been previously reported to be specifically involved in C. jejuni invasion of epithelial cells with no specific involvement in motility (49) and therefore were not considered for further analysis. Twenty-four of the identified genes encode proteins known to be involved in flagellar biosynthesis, modification, regulation, and chemotaxis, which validated the rationale for the screening protocol. Homology searches for the proteins encoded by the remaining 8 genes with a motility defect (CJ81176_0100, CJ81176_0198, CJ81176_0199, CJ81176_0240, CJ81176_0413, CJ81176_0891, CJ81176_1488, and CJ81176_1489) detected no proteins previously associated with flagellar function except for CJ81176_1489. This particular protein exhibits amino acid sequence similarity to FliJ, a poorly conserved component of the flagellar export apparatus, which has been shown to be required for efficient flagellar assembly in S. Typhimurium (43, 50).

Four of the genes showing a motility defect (CJ81176_0100, CJ81176_0198, CJ81176_0199, and
were exclusively detected in Epsilonproteobacteria, including Campylobacter, Helicobacter, Sulfurospirillum, Sulfuricurvum, Sulfurimonas, Wolinella, Caminibacter, and Nautilia (Table 2) (17, 18, 51–57). Genomic localization of the identified putative motility genes revealed that two of them (CJJ81176_0100 and CJJ81176_0891) are located immediately adjacent to known motility genes (see Fig. S1), providing further support for their potential involvement in motility. Taken together, these results indicate that the genetic screen identified several novel putative motility genes.

Functional characterization of the C. jejuni motility mutants. To verify the phenotype of the novel motility mutants, we constructed mutants with deletions of the candidate genes in C. jejuni and subsequently examined their ability to invade cultured mammalian cells. We found a drastic (>100-fold) reduction in the levels of bacterial internalization in cultured mammalian cells in all mutants except for the C. jejuni CJJ81176_0240 mutant, which showed an ~5-fold decrease in invasion (Fig. 2). Since we previously observed that motility defects are always associated with very strong invasion phenotypes (49), it is likely that the invasion defect of the CJJ81176_0240 mutant is due to reasons other than motility and it was therefore not considered any further in our analysis. We used a soft-agar motility plate assay to examine the motility phenotype of all the mutants severely affected in invasion (Fig. 3). Consistent with their cell invasion phenotype, we found that C. jejuni CJJ81176_0100, CJJ81176_0198, CJJ81176_0199, CJJ81176_0413, CJJ81176_0891, and CJJ81176_1488 exhibited a strong motility defect. In contrast, the CJJ81176_1489 mutant showed wild-type motility in this assay, indicating that the invasion defect might be due to factors...
other than motility. Alternatively, its motility defect may not be effectively captured by the motility agar plate assay. In fact, we have previously observed that certain *C. jejuni* mutants showing nearly wild-type motility in the agar plate assay can exhibit a motility phenotype in liquid which can result in severe cultured mammalian cell invasion defects (49). Introduction of a wild-type copy of these genes elsewhere in the chromosome restored the invasion and motility defects to wild-type levels in all but two of the mutants and partially in CJ81176_0100 (see below). The CJ81176_0891 mutant could not be complemented, indicating that its phenotype may be due to polar effects of the mutation on the downstream motility gene *flhA*. In fact, introduction of a wild-type copy of *flhA* into the ΔCJ81176_0891 strain restored its motility (Fig. 3), indicating that the motility defect was due to a polar effect on this downstream motility gene and hence was not analyzed further.

Loss of motility could result from defects in flagellar assembly or defects in the motor that propels flagellar movement. To distinguish between these possibilities, we examined the different *C. jejuni* nonmotile mutants by transmission electron microscopy (TEM) (Fig. 4). The *C. jejuni* ΔCJ81176_1489 mutants showed a complete absence of flagella on the bacterial surface, indicating that this gene is essential for flagellar assembly. This is consistent with its low but significant amino acid sequence similarity to FliJ, which in other systems has been shown to be essential for flagellar assembly (50). Therefore, we propose that CJ81176_1489 is a true

### TABLE 1 Genes identified by INseq exhibiting strong cultured cell invasion phenotype

| 81-176 gene and encoded protein functional category | NCTC11168 gene | Symbol | Annotation | *q* value |
|---------------------------------------------------|----------------|--------|------------|-----------|
| Flagellar assembly                                 |                |        |            |           |
| CJ81176_0097                                      | Cj0059c        | fliY   | Flagellar motor switch protein | 8.39E-09 |
| CJ81176_0098                                      | Cj0060c        | fliM   | Flagellar motor switch protein | 1.44E-19 |
| CJ81176_0101                                      | Cj0063c        | fliG   | ParA family ATPase               | 0.001    |
| CJ81176_0226                                      | Cj0195         | fliI   | Flagellum-specific ATP synthase  | 2.38E-20 |
| CJ81176_0357                                      | Cj0335         | fliB   | Flagellar biosynthesis protein   | 6.27E-16 |
| CJ81176_0358                                      | Cj0336c        | motB   | Flagellar motor protein MotB     | 9.00E-07 |
| CJ81176_0359                                      | Cj0337c        | motA   | Flagellar motor MotA             | 6.21E-19 |
| CJ81176_0837                                      | Cj0820c        | fliP   | Flagellar biosynthesis protein   | 6.63E-30 |
| CJ81176_0860                                      | Cj0862c        | fliA   | Flagellar biosynthesis protein   | 3.14E-08 |
| CJ81176_1044                                      | Cj1025c        | fliQ   | Hypothetical protein             | 0.001    |
| CJ81176_1045                                      | Cj1026c        | fliP   | Putative lipoprotein             | 7.84E-07 |
| CJ81176_1194                                      | Cj1179c        | fliR   | Flagellar biosynthesis protein   | 2.51E-17 |
| CJ81176_1459                                      | Cj1466         | fliK   | Flagellar hook-associated protein| 2.93E-07 |
| CJ81176_1550                                      | Cj1565c        | pflA   | Paralyzed flagellar protein       | 1.22E-13 |
| CJ81176_1671                                      | Cj1675         | fliQ   | Flagellar biosynthesis protein   | 9.94E-08 |
| Regulators for flagellar biosynthesis             |                |        |            |           |
| CJ81176_0099                                      | Cj0061c        | fliA   | Flagellar biosynthesis sigma factor | 1.14E-07 |
| CJ81176_0102                                      | Cj0064c        | fliK   | Flagellar biosynthesis regulator  | 0.001    |
| CJ81176_0696                                      | Cj0670         | rpoN   | RNA polymerase factor sigma54    | 5.77E-15 |
| CJ81176_0814                                      | Cj1024c        | flgR   | Sensor histidine kinase           | 5.43E-24 |
| CJ81176_1043                                      | Cj0795         | flgS   | sigma54-dependent regulator      | 1.16E-07 |
| Flagellar modification                            |                |        |            |           |
| CJ81176_1310                                      | Cj1293         | pseB   | Polysaccharide biosynthesis protein | 8.86E-05 |
| CJ81176_1333                                      | Cj1316c        | pseA   | Flagellin modification protein    | 0.008    |
| Chemotaxis                                        |                |        |            |           |
| CJ81176_0309                                      | Cj0283c        | cheW   | Purine-binding chemotaxis protein | 1.54E-06 |
| CJ81176_0931                                      | Cj0924c        | cheB   | Protein-glutamate methylesterase | 7.29E-04 |
| Genes that were identified in previous studies b   |                |        |            |           |
| CJ81176_0295                                      | Cj0268c        | SPFH domain-containing protein | 0.001 |
| CJ81176_0479                                      | Cj0454c        | Hypothetical protein | 0.001 |
| CJ81176_0481                                      | Cj0456c        | Hypothetical protein | 4.47E-09 |
| CJ81176_0996                                      | Cj0977         | Hypothetical protein | 6.01E-12 |
| Other unknown function                            |                |        |            |           |
| CJ81176_0100                                      | Cj0062c        | Hypothetical protein | 0.004 |
| CJ81176_0198                                      | Cj0162c        | Hypothetical protein | 0.003 |
| CJ81176_0199                                      | Cj0163c        | Hypothetical protein | 2.27E-05 |
| CJ81176_0240                                      | Cj0208         | DNA methyltransferase | 0.003 |
| CJ81176_0413                                      | Cj0390         | TPR domain-containing protein | 7.38E-10 |
| CJ81176_0891                                      | Cj0883c        | ReF2 family protein, putative | 1.62E-06 |
| CJ81176_1488                                      | Cj1496c        | Hypothetical protein | 1.38E-08 |
| CJ81176_1489                                      | Cj1497c        | Hypothetical protein | 2.28E-12 |

a These genes have *q*-value < 0.01.
b These genes were also identified from previous study (49).
TABLE 2 Genes encoding flagellar proteins specific to epsilonproteobacteria identified in this study

| Epsilonproteobacterium | ID of gene corresponding to C. jejuni gene: |
|------------------------|------------------------------------------|
| H. pylori              | HP1358 HP1359 HP1479                      |
| Arcobacter butleri     | NA NA                                      |
| Sulfurospirillum deleyianum | Sulku_0477 Sulku_0283 Sulku_1757         |
| Sulfuricurvum kuijense | Suden_0705 Suden_1809 Suden_1668          |
| Wolinella succinogenes | WS1640 WS0360 NA                         |
| Caminibacter mediaatlanticus | CMTB2_07016 CMTB2_06076 CMTB2_06071 |
| Nautilia profundicola  | NAMH_1348 NAMH_1408 NAMH_1409             |

a The proteins encoded by these genes are found only in epsilonproteobacteria, with no homologs present in any other bacteria. The analysis was done by PSI-BLAST. ID, identifier.

b –, CJJ81176_0100 is not found in H. pylori 26695, but it is present in other H. pylori strains.

c NA, not found in this species.

homolog of FliJ. The ΔCJJ81176_0198 mutant exhibited abnormal flagellation, showing either a lack of flagella or a single flagellum at only one of the bacterial poles (Fig. 4; see also Fig. S2 in the supplemental material), indicating that this gene is required for correct flagellar assembly. In contrast, the ΔCJJ81176_0100, ΔCJJ81176_0199, ΔCJJ81176_0413, and ΔCJJ81176_1488 mutants exhibited apparently normal flagella at their poles, suggesting that these mutations may affect motor function and not flagellar assembly (Fig. 4). In summary, the functional analysis of the candidate genes indicated that at least a subset of them encode potential novel components of the flagellar system.

Subcellular localization of putative flagellar proteins. Because of the complexity of the flagellar system and the energy burden associated with motility, many mutations affecting unrelated physiological process can indirectly affect motility (58, 59). To further ascertain a potential direct role of a subset of the identified motility gene products in flagellar biogenesis or function, we investigated their subcellular localization by fluorescence microscopy. Given the polar localization of flagella in C. jejuni, we reasoned that if the identified proteins play a direct role in flagellar biogenesis and/or its function, they should localized at the cellular poles. We chose to examine CJJ81176_0100, CJJ81176_0413, and
CJJ81176_1488 because the deletion mutants of the genes encoding these proteins exhibited apparently wild-type flagella, did not exhibit amino acid sequence similarity to other flagellar proteins, and/or are not encoded within known flagellar gene loci. We therefore reasoned that additional evidence was necessary to directly implicate these proteins in motility. We constructed C. jejuni strains expressing functional (see Fig. S3 in the supplemental material) green fluorescent protein (GFP)-tagged versions of a subset of the putative flagellar proteins and introduced them into the chromosome at their respective loci by homologous recombination to ensure the native level of expression. We found that the CJ81176_0100, CJ81176_0413, and CJ81176_1488 GFP fusion proteins localized to the two C. jejuni poles (Fig. 5). This observation further supports the idea of a potential direct role of these proteins in flagellar structure, assembly, and/or function.

Interaction of putative flagellar proteins with known flagellar components. To provide additional evidence for a potential direct role in the motility of the proteins identified in our screen, we searched for interacting proteins with the goal of identifying interactions with known flagellar components. We reasoned that identifying interactions with known flagellar proteins would further support the idea of a direct role in flagellar function and/or assembly. We generated functional, FLAG-tagged fusion constructs of the identified putative flagellar proteins and introduced them into the C. jejuni chromosome by allelic exchange. We then identified interacting proteins by coimmunoprecipitation (co-IP) followed by liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Protein interactions were further confirmed by constructing C. jejuni strains encoding differentially tagged versions of the interacting proteins and subsequently examining the strains by immunoprecipitation and Western blot analysis (Table 3 and Fig. 6).

We found that CJ81176_0100 interacts with FliF (Table 3 and Fig. 6), which is a central component in the flagellar membrane and supramembrane ring located in the inner membrane (60). Although our results cannot address whether the interaction of CJ81176_0100 and FliF is direct or indirect, it is noteworthy that CJ81176_0100 has two putative transmembrane domains predicting its location at the inner membrane, where it could potentially interact directly with FliF. The idea of the potential physical interaction of CJ81176_0100 with the flagellar apparatus is also supported by the observation that, although less consistently, interactions with other flagellar structural components such as FlhA, FlgC, and FlgB were also detected, suggesting that, perhaps through FliF, CJ81176_0100 may interact with the flagellar apparatus. It is not clear how CJ81176_0100 contributes to motility, but it is intriguing that our analysis of CJ81176_0100-interacting proteins also identified CJ81176_0996, a sigma28 (FliA)-regulated protein (61, 62). In fact, coimmunoprecipitation experiments using FLAG-tagged CJ81176_0996 as an affinity probe detected CJ81176_0100 and FliF as interacting proteins, further supporting the idea of the interaction among these flagellar proteins (Table 3 and Fig. 7). It has been reported that a mutation in CJ81176_0996 results in a drastic defect in C. jejuni entry into cultured mammalian cells (61) (confirmed in our screen; see Table 1), which was later shown to be due to a motility defect manifested in liquid cultures but not on motility plates (49). These
observations provide support for the idea of a direct role in motility for both CJJ81176_0100 and CJJ81176_0996.

Our analysis showed that CJJ81176_0198 interacts with FliO (Table 3 and Fig. 6), a component of the flagellar type III secretion export apparatus (63), thus supporting the idea of a direct role for CJJ81176_0198 in motility. This finding is consistent with the observation that the C. jejuni ΔCJJ81176_0198 mutant exhibited aberrant or defective flagellar assembly (see Fig. 4).

CJJ81176_0199 showed interactions with MotA and MotB (Table 3 and Fig. 6), two components of the flagellar motor (64). Consistent with this interaction, the C. jejuni ΔCJJ81176_0199 mutant exhibited apparently normal flagella although it is non-motile. Therefore, CJJ81176_0199 may exert its effect by modulating motor assembly or function.

We found that CJJ81176_0413 interacts with PflA, a protein exclusively encoded by Epsilonproteobacteria (65) and previously shown to be required for motility (46). Interestingly, disruption of pflA results in apparently normal but paralyzed flagella (46), the same phenotype observed after the disruption of CJJ81176_0413. Electron microscopy examination of the C. jejuni ΔCJJ81176_0413 mutant showed apparently normal flagella at both poles (Fig. 4) although the mutant was completely defective in motility (Fig. 3). These observations suggest a role for these proteins in the function and/or assembly of the flagellar motor. Despite a lack of detectable primary amino acid sequence similarity between CJJ81176_0413 and PflA, structural homology searches indicated that these proteins share structural similarities to the same O-linked N-acetylglucosaminyltransferase (PDB 1W3B [66]), suggesting that these two proteins are structurally similar. C. jejuni flagellin and other flagellar components are known to be glycosylated, a modification that is required for flagellar assembly (31, 67–69). It is possible that CJJ81176_0413 as well as PflA may be involved in this process. Intriguingly, both these proteins have tetratricopeptide (TPR) repeats, which previous studies have implicated in conferring substrate specificity to eukaryotic N-acetylglucosaminyltransferases (70). The presence of these repeats in CJJ81176_0413 as well as PflA is consistent with a potential role in protein glycosylation. Interestingly, CJJ81176_0413 also interacted with KdpD and CJJ81176_1442, the latter of which belong to a cluster of genes implicated in the biosynthesis of the capsular polysaccharide of C. jejuni (71), suggesting that its activity may not be exclusively associated with flagellar biosynthesis.

Lastly, we detected interactions of CJJ81176_1488 with FlgM and FliK (Table 3 and Fig. 6), two conserved essential components of the flagellar system. FlgM is an anti-sigma factor that controls expression of class 3 flagellar genes by directly binding the flagellum-specific transcription factor sigma28 (FliA), thus preventing the expression of genes controlled by this regulator (72, 73). FliK, on the other hand, is involved in the regulation of substrate switching during flagellar assembly (74). Although these flagellar proteins exhibit very different functions, they are both secreted through the flagellar type III secretion system. In this context, it is noteworthy that CJJ81176_1488 showed polar localization. Since FlgM would likely be cytoplasmic when interacting with sigma factors, it is likely that CJJ81176_1488 interacts with FlgM only when it is being secreted and not when it is undertaking its regulatory functions. We therefore hypothesize that CJJ81176_1488 may assist in some aspect of flagellar type III secretion. Consistent with this hypothesis, upstream of CJJ81176_1488 is CJJ81176_1489, which we propose is the homolog of FliI, a cytoplasmic component of the flagellar type III export apparatus involved in flagellar protein export. Although the ΔCJJ81176_1488 mutant retains some motility, its subcellular localization, genomic organization, and interactome support the idea of a direct role for this protein in flagellar biology.

**DISCUSSION**

Flagella are widely distributed organelles among bacterial species. Although the basic architecture and core components of this organelle are highly conserved, it is becoming increasingly clear that there are significant differences among flagellar structures from different bacterial species. Among the more structurally diverse flagella are those of Epsilonproteobacteria such as C. jejuni and H. pylori. Indeed, previous cryo-electron tomography studies have visualized specific protein densities in the cryotomograms of C. jejuni and H. pylori flagella that must correspond to unique components of this organelle in these bacteria (12). Most of the knowledge on bacterial flagella is derived from studies done in S. Typhimurium and E. coli. However, since the flagellar structures of these bacteria are among the simplest, the annotation of flagellar genes in bacterial genomes based on homologies to these...
model organisms is likely to miss essential flagellar components in other bacterial species with more-complex flagella. We have described here several novel C. jejuni flagellar genes identified through a comprehensive high-throughput genetic screening. Although some mutations could indirectly lead to lack of motility, through a variety of functional, biochemical, and in vivo imaging analyses we have provided strong evidence that the genes we have identified encode factors directly involved in the assembly and/or function of the flagellar apparatus. Consequently, we have assigned to these different proteins a nomenclature consistent with their newly identified role in motility (Fig. 8; see also Table S3 in the supplemental material). Thus, we have renamed CJ81176_100 FlgV, CJ81176_0998 FlgW, CJ81176_0199 FlgX, CJ81176_1448 FlgY, CJ81176_0413 PflB, and CJ81176_1489 FliJ.

Our analysis identified 6 new components of the flagellar system which, on the basis of the phenotypes of the mutants and their interactome, we postulate play different roles in motility or flagellar assembly. Of these proteins, only CJ81176_0100 is located within a known flagellar gene cluster, which contains flhF, flhG, fliA, fliY, and fliM. Therefore, it is likely that CJ81176_0100 is coregulated with these flagellar genes. However, none of the other

### TABLE 3 Proteins that interact with the flagellar proteins identified in this study

| Gene ID          | Symbol | Annotation                        | Protein mass (Da) | No. of spectral counts | R1 | R2 | R3 |
|------------------|--------|-----------------------------------|-------------------|------------------------|----|----|----|
| CJ81176_0100     | FlgV   | Hypothetical protein              | 14221             | 17                     | 1  | 1  | 1  |
| CJ81176_0340     | FliF   | Flagellar membrane and supramembrane-ring protein | 62579             | 153                    | 4  | 136|     |
| CJ81176_0998     | Hypothetical protein              | 21265             | 44                    | 8                       | 30 |     |
| CJ81176_1732     | Hypothetical protein              | 13124             | 31                    | 27                      |    |     |
| CJ81176_0890     | FlhA   | Flagellar biosynthesis protein    | 77538             | 11                     | 12 |     |
| CJ81176_0552     | FlgC   | Flagellar basal body rod protein  | 18317             | 3                      | 6  |     |
| CJ81176_0553     | FlgB   | Flagellar basal body rod protein  | 16055             | 4                      | 2  |     |
| CJ81176_0433     | FrdA   | Fumarate reductase subunit        | 74653             | 2                      | 2  |     |
| CJ81176_0996     | Hypothetical protein              | 21265             | 47                    | 62                     | 14 |     |
| CJ81176_1732     | Hypothetical protein              | 13124             | 21                    | 6                       | 8  |     |
| CJ81176_0340     | FliF   | Flagellar membrane and supramembrane-ring protein | 62579             | 1                      | 2  | 4  |     |
| CJ81176_0100     | Hypothetical protein              | 14221             | 4                     | 4                       | 1  |     |
| CJ81176_0757     | GjaC   | GjaC protein                      | 27838             | 2                      | 3  | 2  |     |
| CJ81176_1205     | CetA   | Methyl-accepting chemotaxis protein | 51156             | 3                      | 5  |     |
| CJ81176_0376     | FlgO   | Flagellar export apparatus protein | 31089             | 46                     | 6  | 51 |     |
| CJ81176_0289     | MacA   | Macrolide-specific efflux protein | 72546             | 18                     | 10 |     |
| CJ81176_0635     | MeltA  | Methyl-accepting chemotaxis protein | 42778             | 28                     | 20 |     |
| CJ81176_0168     | CetA   | Methyl-accepting chemotaxis protein | 51156             | 14                    | 1  | 11 |     |
| CJ81176_1205     | CetA   | Methyl-accepting chemotaxis protein | 40562             | 9                      | 4  |     |
| CJ81176_0971     | CetA   | Carbon-nitrogen family hydrolase  | 34107             | 5                      | 6  |     |
| CJ81176_1272     | Hypothetical protein              | 25530             | 4                      | 6  |     |     |
| CJ81176_0412     | SerS   | Seryl-tRNA synthetase             | 46900             | 2                      | 1  | 2  |     |
| CJ81176_0199     | Hypothetical protein              | 7859              | 22                    | 12                     | 51 |     |
| CJ81176_0359     | MotA   | Flagellar motor protein           | 28320             | 15                     | 2  |     |     |
| CJ81176_0358     | MotB   | Flagellar motor protein           | 27880             | 3                      | 1  |     |     |
| CJ81176_1204     | CetB   | Methyl-accepting chemotaxis protein | 19358             | 5                     | 4  |     |     |
| CJ81176_0507     | RplJ   | 50S ribosomal protein L10         | 17761             | 3                      | 4  |     |     |
| CJ81176_0920     | CysK   | Cysteine synthase A               | 27208             | 1                      | 2  | 2  |     |
| CJ81176_1452     | Hypothetical protein              | 42424             | 3                      | 2  |     |     |
| CJ81176_0413     | TPR domain-containing protein     | 93554             | 609                   | 367                     | 444|     |
| CJ81176_1550     | PiLA   | Paralyzed flagellar protein       | 91269             | 133                    | 57 | 241|     |
| CJ81176_1442     | Hypothetical protein              | 15629             | 33                    | 8                       | 7  |     |     |
| CJ81176_0702     | KdpD   | Truncated KdpD protein            | 73235             | 3                      | 2  | 1  |     |
| CJ81176_0920     | CysK   | Cysteine synthase A               | 27208             | 2                      | 2  |     |     |
| CJ81176_1488     | Hypothetical protein              | 19620             | 91                    | 30                     | 210|     |
| CJ81176_1457     | FlgM   | Anti-sigma factor protein         | 7139              | 2                      | 1  | 20 |     |
| CJ81176_0079     | FlhK   | Flagellar substrate switch protein | 69078             | 3                      | 2  | 15 |     |
| CJ81176_1205     | CetA   | Methyl-accepting chemotaxis protein | 31156             | 2                      | 3  | 210|     |

*a The bait proteins are highlighted in boldface characters. Only detected protein with spectral counts > 1 in at least 2 replicates after filtering the proteins that were also detected in control sample are presented here. Data represent the results of three independent experiments.

*b R, replicate. For replicate 2, analysis of the Co-IP proteins was conducted under more-stringent conditions resulting in lower spectral counts.

*c The protein encoded by the CJ81176_0100 gene is a membrane protein which is more difficult to detect by MS/MS, therefore resulting in lower spectral counts.
identified genes are located within known flagellar gene clusters and there are no previous reports indicating that they are coregulated by a known flagellar regulator such as RpoN, FliA, or FlgRS. One of the identified proteins, CJ81176_1489, was shown to be essential for flagellar assembly since its mutation resulted in no flagella on the bacterial surface. This is consistent with the observation that CJ81176_1489 exhibits low but significant amino acid sequence similarity to FliJ, a poorly conserved flagellar protein shown to be essential for flagellar assembly in *S. Typhimurium*. Another protein identified in this study, CJ81176_0198, was also shown to be required for proper flagellar assembly, and a *C. jejuni* strain carrying a mutation in this gene exhibited either no flagella or a flagellum at only one of the poles instead of at the two poles as seen in the wild type. Consistent with a role in flagellar assembly, CJ81176_0198 was shown to interact with FliO, a component of the flagellar export apparatus.

The rest of the identified proteins (CJJ81176_0100, CJ81176_0199, CJ81176_0413, and CJ81176_1488) are not essential for flagellar assembly since wild-type flagella were identified on the surface of the respective mutant strains. Consequently, we postulate that they play a role in motor function. Consistent with this role, CJ81176_0199 was shown to interact with the motor proteins MotA and MotB. Since no homolog of this protein exists other than in *Epsilonproteobacteria*, it is likely that this protein performs a function unique to the flagella of this group of bacteria. Cryo-electron tomography studies have shown unique protein densities in the flagellar structure of *Epsilonproteobacteria* which have been proposed to be associated in part with unique structural features of its motor. It is possible that CJ81176_0199 and the other flagellar proteins unique to epsilon proteobacteria identified in this study may account for some of those unique protein densities. It is not clear how the other identified proteins may exert their function, but imaging analysis located them at the bacterial poles. It is intriguing that CJ81176_1488 interacts with FlgM and FliK, two proteins that are exported by the flagellar apparatus. It is therefore possible that CJ81176_1488 has a role in coordinating protein secretion. CJ81176_0100, on the other hand, was shown to interact with FliF, a central component of the flagellar basal body. How CJ81176_0100 exerts its function is not...
clear, although its location and interactome and the phenotype of its mutation suggest a role in motility but not in flagellar assembly. In addition, our interactome studies indicate that CJ81176_0996 may play a direct role in motility by interacting with CJ81176_0100 and FlhF.

One of the unique features of the Epsilonproteobacteria flagella is that some of the components are posttranslationally modified by specific glycosyltransferases. Although the role of glycosylation in flagellar function is not understood, it is clear that, at least in the case of flagellin, protein glycosylation is necessary for flagellar assembly. In this context, it is intriguing that one of the identified proteins in our study, CJ81176_0413, exhibits amino acid sequence similarity to glycosyl transferases. Furthermore, our interactome study showed that this protein interacts with PipA, a previously identified protein required for motility. In fact, structure-based homology searches indicate that, despite their low primary amino acid sequence identity, CJ81176_0413 and PipA share obvious structural similarities. Therefore, it is possible that both these proteins exert a related function and that this function may involve the posttranslational modification of some flagellar component. Given its interactome and structural similarity, we have renamed CJ81176_0413 PipB.

Through the most comprehensive genetic screen yet to be performed in C. jejuni coupled to functional, biochemical, and imaging analysis, we have identified novel flagellar components that are widely distributed among but restricted to Epsilonproteobacteria. The identification of these components provides the basis to understand the specific adaptation of this remarkable bacterial organelle to function in the context of a widely diverse set of bacterial species and brings unique insight into its evolution and function.

**MATERIALS AND METHODS**

**Bacterial strains, cell lines, and culture conditions.** The complete list of strains and plasmids used in this study is shown in Table S4 in the supplemental material. The C. jejuni 81-176 wild-type strain was grown on brucella broth agar or on blood agar plates (Trypticase soy agar supplemented with 5% sheep blood) at 37°C in an incubator equilibrated to a 10% CO₂ atmosphere. The C. jejuni transfectants were selected on plates supplemented with 50 μg/ml kanamycin and/or 7.5 μg/ml chloramphenicol, as indicated below. For liquid cultures, C. jejuni strains were grown in brain heart infusion (BHI) medium with no antibiotics added. All C. jejuni strains were stored at −80°C in BHI broth containing 30% glycerol.

Cos-1 (African green monkey kidney fibroblast-like cell line) cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). All cell lines were kept under a 5% CO₂ atmosphere.

**Construction of C. jejuni transposon mutant library.** The transposon used in this study is a derivative of the Himar1 Mariner transposon with a single nucleotide change to result in an Mmel site in each inverted repeat (37). To minimize potential transcriptional polar effects of the insertions, two genes encoding kanamycin (from pILL600) and chloramphenicol (from pRY109) resistance lacking transcription terminators were cloned within the transposable element with their promoters facing the insertions, two genes encoding kanamycin (from pILL600) and chloramphenicol (from pRY109). Resistance lacking transcription terminators were cloned within the transposable element with their promoters facing outward to promote the transcription of downstream genes and minimize the possibility of polar effects (Fig. 1A). The transposon was constructed by recombinant PCR amplification of both antibiotic resistance genes using primers listed in Table S5 in the supplemental material and was introduced into pBluescript II SK plasmid, resulting in the final plasmid, pSB4118.

An *in vitro* transposon mutagenesis system was used to generate insertion mutants of *C. jejuni* as previously described (40). Briefly, the *in vitro* transposition reactions were carried out with purified MarC9 transposase, C. jejuni DNA, and transposon plasmid pSB4118 with reaction buffers described previously (38, 75). Reaction mixtures were incubated for 4 h at 30°C and inactivated for 20 min at 72°C, and the transposition products were transformed into naturally competent *C. jejuni* and transformants plated on brucella agar plates containing both kanamycin and chloramphenicol. After 48 h of incubation, the transformants were collected from plates and pooled into cryo tubes with approximately 5,000 transformants per tube. A total of ~50,000 transformants were collected.

**Screening of C. jejuni transposon mutants for their ability to enter cultured cells.** The screening of the *C. jejuni* transposon insertion library for mutants unable to enter cultured Cos-1 cells was carried out as previously described (49). To determine the size of the potential “bottleneck” of the assay, bacterial infections were carried out with different ratios of two differently marked strains of *C. jejuni* that have equal cultured-cell invasion capacities. This test showed that for a 10-cm-diameter dish infected with a multiplicity of infection (MOI) of 100, even a ratio of 1:16,000 allowed the detection of both strains without any measurable stochastic loss. Based on these results, a mutant pool of 5,000 mutants was used to infect each 10-cm-diameter dish infection, which ensured that no mutant would be stochastically lost during the infection assay. Ten mutant pools of 5,000 were screened as one biological replicate totaling ~50,000 mutants. A total of three independent biological replicates, each with ~50,000 mutants, were carried out to ensure the robust coverage of the library.

**INseq DNA sample preparation and data analysis.** The INseq DNA sample preparation and amplification were carried out as previously described (37, 48). The resulting 125-bp products from the mutant pools were sequenced on an Illumina HiSeq2000 system at the Yale Center for Genomic Analysis. The sequencing data were analyzed using the INseqpipeline_v2 package (48). The processed data are provided in Table S1 in the supplemental material. Essential genes were identified using the R package *Negenes* (76) as previously described (37) with two modifications: insertions represented by (i) fewer than 3 counts per million reads or (ii) insertions in the distal 20% of each gene were excluded from the analysis.

**C. jejuni mutant strain construction.** C. jejuni 81-176 knockout mutant strains were constructed by PCR amplification of the flanking regions of these open reading frames (ORFs) with specific primers (see Table S5 in the supplemental material) and cloning of a kanamycin resistance cassette (*aphA3*) between the amplified flanking regions. The resulting plasmids (built on a pBluescript II SK backbone) were used to move the mutated alleles into the chromosome of *C. jejuni* 81-176 by natural transformation and allelic recombination. Complementation of the mutant strains of *C. jejuni* was achieved by introducing a wild-type copy of the gene at the *hsdM* locus as previously described (77). Briefly, the gene with its original Shine-Dalgarno sequence was cloned into pSB3313 to generate a 3×FLAG fusion protein, which is expressed from a cat promoter upstream, at the C terminus. The resulting 3×FLAG fusion protein was moved into pSB3021, which was then integrated into the *hsdM* locus of the chromosome where its expression is driven by a chloramphenicol resistance gene promoter (77). To confirm the protein interactions identified by LC-MS/MS, *C. jejuni* strains were constructed in which the interacting proteins were tagged with different epitopes. Briefly, the genes encoding the identified interacting proteins (*Flf, fliO, motA, motB, pFlA, fliK, and fliM*) were cloned into pSB4868 to generate 454-tagged versions of these proteins. 454-tagged genes were then cloned into pSB3021-derived plasmids carrying FLAG-tagged versions of the genes encoding the corresponding interacting motility proteins identified in the mutant strain. The resulting plasmids were then integrated into the *hsdM* locus of the *C. jejuni* chromosome as described above (see Table S4).

**Motility plate assay and EM imaging.** The optical density at 600 nm (OD600) of the bacterial cultures to be tested was adjusted to 0.4 and spotted onto soft agar (0.8% [wt/vol]). Plates were incubated for 24 h at 37°C, and the swarming diameter of the tested strain was compared to those of the wild-type strain and the nonmotile *C. jejuni ΔmotA* mutant strain. Bacterial flagella were visualized by negative staining and transmis-
sion electron microscopy (TEM). Briefly, bacterial cells were pelleted (2,000 rpm for 2 min) and resuspended in prewarmed phosphate-buffered saline (PBS). Samples were directly applied to glow-discharged carbon-coated 200-mesh Cu grids and stained using 2% phosphotungstic acid (pH 7.0). Images were acquired using 10,000-fold to 35,000-fold magnification on a Tecnai Biotwin TEM (FEI Company) at 80 kV. Images were collected using a Morada Soft Imaging system and a 6-M-pixel charge-coupled-device (CCD) camera (Olympus).

Fluorescence microscopy. Fusions of selected C. jejuni proteins to the amino terminus of superfolder GFP (sfGFP) were introduced at the native loci by homologous recombination using standard recombinant DNA techniques and natural transformation. A strain expressing FLAG- and M45-tagged versions of the interacting proteins were grown and lysed as indicated above. Immunoprecipitations were performed using the sfGFP strain encoding FlhF and FlhG in the Vibrio cholerae flagellar transcription hierarchy. J. Bacteriol. 187:6324–6332. http://dx.doi.org/10.1128/JB.187.16.6324-6332.2005.

Mc CARTER LL. 2004. Dual flagellar systems enable motility under different circumstances. J. Mol. Microbiol. Biotechnol. 7:18–29. http://dx.doi.org/10.1159/000078766.

Atsumi T, McCarter I, Imae Y. 1992. Polar and lateral flagellar motors of marine Vibrio are driven by different ion-motive forces. Nature 355:182–184. http://dx.doi.org/10.1038/355182a0.

Z hao X, Zhang K, Bouqui T, Hu B, Motaleb MA, Miller KA, James ME, Charon NW, Manson MD, Norris SJ, Li C, Liu J. 2013. Cryoelectron tomography reveals the sequential assembly of bacterial flagella in Borrelia burgdorferi. Proc. Natl. Acad. Sci. U. S. A. 110:14390–14395. http://dx.doi.org/10.1073/pnas.1308306110.

Macnab RM. 2003. How bacteria assemble flagella. Annu. Rev. Microbiol. 57:77–100. http://dx.doi.org/10.1146/annurev.micro.57.030502.005832.

Snyder LA, Loman NJ, Füterer K, Pallen MJ. 2009. Bacterial flagellar diversity and evolution: seek simplicity and distrust it! Trends Microbiol. 17:51–60. http://dx.doi.org/10.1016/j.tim.2008.10.002.

Liu R, Ochman H. 2007. Stepwise formation of the bacterial flagellar system. Proc. Natl. Acad. Sci. U. S. A. 104:7116–7121. http://dx.doi.org/10.1073/pnas.0700266104.

Chen S, Beeby M, Murphy GE, Leadbetter JR, Hendrixson DR, Briegel B, Berg HC. 2003. Characterization of an autotrophic sulfide-oxidizing marine Arcobacter sp. that produces filamentous sulfur. Appl. Environ. Microbiol. 68:316–325. http://dx.doi.org/10.1128/AEM.68.1.316-325.2002.

Labrenz M, Grote J, Mammitzsch K, Boscker HT, Laue M, Just G, Glauhitz S, Jürgens K. 2013. Sulfitomonas goetlandica sp. nov., a chemotrophic and psychrotolerant epilithonbacteria isolated from a pelagic Baltic Sea redoxcline, and an emended description of the genus Sulfitomnas. Int. J. Syst. Evol. Microbiol. 63:4141–4148. http://dx.doi.org/10.1099/ijs.0.048827-0.

Cueff V, Cambon-Bouvatia MA. 2002. Caminibacter hydrogeniphilus gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent. Int. J. Syst. Evol. Microbiol. 52:1317–1329. http://dx.doi.org/10.1099/ijs.0.02195-0.

Sikorski J, Lapidus A, Copeland A, Glavina Del Rio T, Nolan S, Lucas S, Chen F, Tice H, Cheng JF, Saunders E, Bruce D, Goodwin L, Pitluck S, Oschinnikova G, Pati A, Ivanova N, Movamrtos K, Chen A, Palaniappan K, Chain P, Land M, Hauser L, Chang YJ, Jeffries CD, Brettin T, Detter JC, Han C, Rohde M, Lang E, Spring S, Göker M, Bristow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk HP. 2010. Complete genome sequence of Sulfurospirillum delenosum type strain (5175). Stand. Genomic Sci. 5:219–157. http://dx.doi.org/10.4056/sigs.671209.

A lain K, Querelle G, Lesongeur F, Pignet P, Crassous P, Rahmzès G, Cuffe V, Cambon-Bonavita MA. 2002. Caminibacter hydrogeniphilus gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent. Int. J. Syst. Evol. Microbiol. 52:1317–1329. http://dx.doi.org/10.1099/ijs.0.02195-0.

Sikorski J, Lapidus A, Copeland A, Glavina Del Rio T, Nolan S, Lucas S, Chen F, Tice H, Cheng JF, Saunders E, Bruce D, Goodwin L, Pitluck S, Oschinnikova G, Pati A, Ivanova N, Movamrtos K, Chen A, Palaniappan K, Chain P, Land M, Hauser L, Chang YJ, Jeffries CD, Brettin T, Detter JC, Han C, Rohde M, Lang E, Spring S, Göker M, Bristow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk HP. 2010. Complete genome sequence of Sulfurospirillum delenosum type strain (5175). Stand. Genomic Sci. 5:219–157. http://dx.doi.org/10.4056/sigs.671209.

Han C, Kotsurzenko O, Chertkov O, Held B, Lapidus A, Nolan S, Lucas S, Hammon N, Deshpande S, Cheng JF, Tapia R, Goodwin LA, Pitluck S, Liolios K, Packan I, Ivanova N, Movamrtos K, Mikhailova N, Pati A, Chen A, Palaniappan K, Land M, Hauser L, Chang YJ, Jeffries CD, Blazcsovics EM, Rohde M, Spring S, Sikorski J, Göker M, Woyte Y, Brettow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk HP, Detter JC. 2012. Complete genome sequence of the sulfur compounds oxidizing chemolithoautotrophic Sulforicurvum kujiiense type strain (YK-1(T)). Stand. Genomic Sci. 6:94–103. http://dx.doi.org/10.4056/sigs.2456004.

Pérez-Rodríguez I, Ricci J, Voordeckers JW, Starovoytov V, Vetrani C.

Epsilonproteobacterium Flagellar System Components
Frirdich E, Biboy J, Adams C, Lee J, Ellermeier J, Gielda LD, Dirita VJ, Salama NR, Hartung ML, Müller A, Hendrixson DR, DiRita VJ. 2009. Tn-seq: high-throughput in silico determination of essential genes of Campylobacter jejuni. J. Bacteriol. 183:2384–2388. http://dx.doi.org/10.1128/JB.183.7.2384-2388.2001

Stahl M, Stintzi A. 2011. Identification of essential genes in C. jejuni genome highlights hyper-variable plasticity regions. Funct. Integr. Genomics 11:241–257. http://dx.doi.org/10.1007/s10202-011-0214-7.

Metris A, Reuter M, Gaskin DJ, Baranyi J, van Vliet AH. 2011. In vivo and silico determination of essential genes of Campylobacter jejuni. BMC Genomics 12:535. http://dx.doi.org/10.1186/1752-2431-12-535.

Pallen MJ, Penn CW, Chauhduri RR. 2005. Prevalence of Campylobacter jejuni in the post-genomic era. Trends Microbiol. 13:143–149. http://dx.doi.org/10.1016/j.tim.2005.02.008.

26. Gao et al. 2009. Life in the human stomach: persistence strategies of the bacterial pathogen Helicobacter pylori. Nat. Rev. Microbiol. 7:505–516. http://dx.doi.org/10.1038/nrmicro2106.

27. Hendrixson DR, DiRita VJ. 2000. Identification of Campylobacter jejuni genes involved in commensal colonization of the chick gastrointestinal tract. Mol. Microbiol. 32:471–484. http://dx.doi.org/10.1046/j.1365-2958.2000.01980.x.

28. Guerry P. 2007. Campylobacter flagella: not just for motility. Trends Microbiol. 15:456–461. http://dx.doi.org/10.1016/j.tim.2007.09.006.

29. Ottemann KM, Lowenthal AC. 2002. Helicobacter pylori uses motility for initial colonization and to attain robust infection. Infect. Immun. 70:1984–1990. http://dx.doi.org/10.1128/IAI.70.4.1984-1990.2002.

30. Lersethakkarn P, Ottemann KM, Hendrixson DR. 2011. Motility and chemotaxis in Campylobacter and Helicobacter. Annu. Rev. Microbiol. 65:389–410. http://dx.doi.org/10.1146/annurev-micro-090310-102908.

31. Gilbreath JJ, Cody WL, Merrell DS, Hendrixson DR. 2010. N-linked protein glycosylation in a bacterial system. Methods Mol. Biol. 600:428–435. http://dx.doi.org/10.1007/886-4-1113-497-1.

32. Schirm M, Soo EC, Aubry AJ, Logan SM. 2005. Stable accumulation of sigma54 in Helicobacter pylori. Mol. Microbiol. 57:1458–1471. http://dx.doi.org/10.1111/j.1365-2958.2005.04900.x.

33. Pereira L, Hoover TR. 2005. Flagellar protein export in Salmonella. J. Bacteriol. 187:4463–4469. http://dx.doi.org/10.1128/JB.187.11.4463-4469.2005.

34. Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone CA, Knight R, Gordon JI. 2009. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe 6:279–289. http://dx.doi.org/10.1016/j.chom.2009.08.003.

35. van Opijnen T, Bodi KL, Camilli A. 2009. Seq-seq: high-throughput parallel sequencing for fitness and genetic interaction studies in microorganisms. Nat. Methods 6:767–772. http://dx.doi.org/10.1038/nmeth.1377.
bacterium isolated from a deep-sea hydrothermal vent. Stand. Genomic Sci. 5:135–143. http://dx.doi.org/10.4056/sigs.2094859.

57. Campbell BJ, Smith JL, Hanson TE, Klotz MG, Stein LY, Lee CK, Wu D, Robinson JM, Khouri HM, Eisen JA, Cary SC. 2009. Adaptations to submarine hydrothermal environments exemplified by the genome of Nautilus profundicola. PLoS Genet. 5:e1000362. http://dx.doi.org/10.1371/journal.pgen.1000362.

58. Nielubowicz GR, Smith SN, Mobley HL. 2010. Zinc uptake contributes to motility and provides a competitive advantage to Proteus mirabilis during experimental urinary tract infection. Infect. Immun. 78:2823–2833. http://dx.doi.org/10.1128/IAI.01220-09.

59. Goon S, Ewing CP, Lorenzo M, Pattarini D, Majam G, Guerry P. 2014. A sigma28-regulated nonflagellar gene contributes to virulence of Campylobacter jejuni 81–176. Infect. Immun. 82:769–772. http://dx.doi.org/10.1128/IAI.01365-2958.2010.07079.x.

60. Grünenefelder B, Gehrig S, Jenal U. 2003. Role of the cytoplasmic C terminus of the FliF motor protein in flagellar assembly and rotation. J. Bacteriol. 185:1624–1633. http://dx.doi.org/10.1128/JB.185.5.1624-1633.2003.

61. Goon S, Ewing CP, Lorenzo M, Pattarini D, Majam G, Guerry P. 2006. A sigma28-regulated nonflagella gene contributes to virulence of Campylobacter jejuni. Mol. Microbiol. 51:1817–1826. http://dx.doi.org/10.1111/j.1365-2958.2003.03954.x.

62. Yokoyama T, Paek S, Ewing CP, Guerry P, Yeo HJ. 2008. Structure of a sigma28-regulated nonflagellae virulence protein from Campylobacter jejuni. J. Mol. Biol. 384:364–376. http://dx.doi.org/10.1016/j.jmb.2008.09.036.

63. Barker CS, Meshcheryakova IV, Kostyukova AS, Samatey FA. 2010. FIoI regulation of FliP in the formation of the Salmonella enterica flagellum. PLoS Genet. 6:e1001143.

64. Muramoto K, Macnab RM. 1998. Deletion analysis of MotA and MotB, components of the force-generating unit in the flagellar motor of Salmonella. Mol. Microbiol. 29:1191–1202. http://dx.doi.org/10.1046/j.1365-2958.1998.00998.x.

65. Gupta RS. 2006. Molecular signatures (unique proteins and conserved indels) that are specific for the Epsilon Proteobacteria (Campylobacteraceae). BMC Genomics 7:167. http://dx.doi.org/10.1186/1471-2164-7-167.

66. Jinek M, Rehwinkel J, Lazarus BD, Izaurralde E, Hanover JA, Conti E. 2004. The superhelical TPR-repeat domain of O-linked GlcNAc transferase exhibits structural similarities to importin alpha. Nat. Struct. Mol. Biol. 11:1001–1007. http://dx.doi.org/10.1038/nsmb883.

67. Alemka A, Nothaft H, Zheng J, Szymanski CM. 2013. N-glycosylation of Campylobacter jejuni surface proteins promotes bacterial fitness. Infect. Immun. 81:1674–1682. http://dx.doi.org/10.1128/IAI.01370-12.

68. Szymanski CM, Logan SM, Linton D, Wren BW. 2003. Campylobacter—A tale of two protein glycosylation systems. Trends Microbiol. 11:233–238. http://dx.doi.org/10.1016/S0966-842X(03)00079-9.