Phenotypic Analysis of the fibronectin binding proteins CadF and FlpA of *Campylobacter jejuni*

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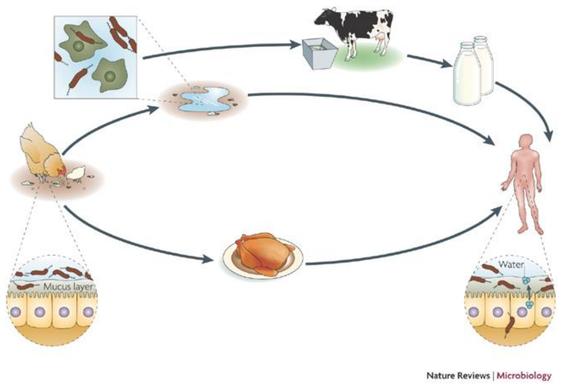
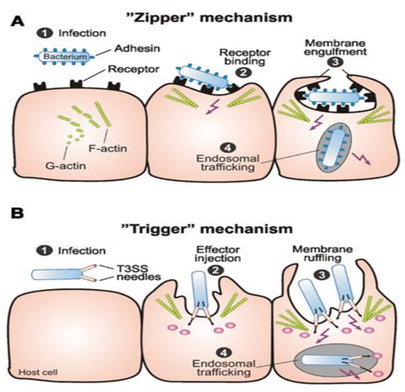
INTRODUCTION

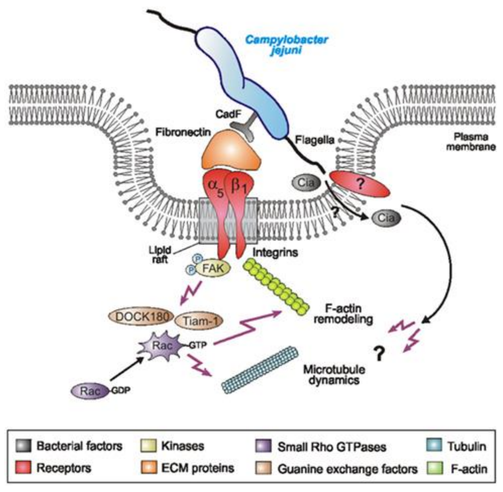
Campylobacter jejuni is a proteobacteria that is a commensal organism in chickens and other avian species. *C. jejuni* can be passed from contaminated food products to humans, where it invades the epithelial cells of the gut and causes disease.



Adherence to and invasion of epithelial cells in humans causes disease, such as diarrhea and inflammation of the intestine.



In order to adhere to and invade cells, *C. jejuni* uses fibronectin-binding proteins to attach to fibronectin in the extracellular matrix of host cells. CadF and FlpA are two such fibronectin-binding proteins, or adhesins, which were the focus of this study.



The goal of this study was to determine the importance of CadF and FlpA proteins in agglutination and cell invasion by studying bacteria lacking these proteins.

METHODS

- SDS-page protein gel run at 100v of bacterial cell lysates of 11168 and 81176 *C. jejuni* strains, WT and mutants.
- Coomassie stain and Western blot of SDS-page gels
- Agglutination assays in PBS, LB, and RPMI. Agglutination was quantified by calculating the change in optical density of the top layer of the sample over a 24 hour period.
- An invasion assay of HCT-8 epithelial cells with *C. jejuni* 11168 and 81176 WT and adhesin mutants. After a 4 hour incubation, cells were fixed and visualized by fluorescent microscopy with staining with DAPI and an anti-*C. jejuni* antibody.

RESULTS

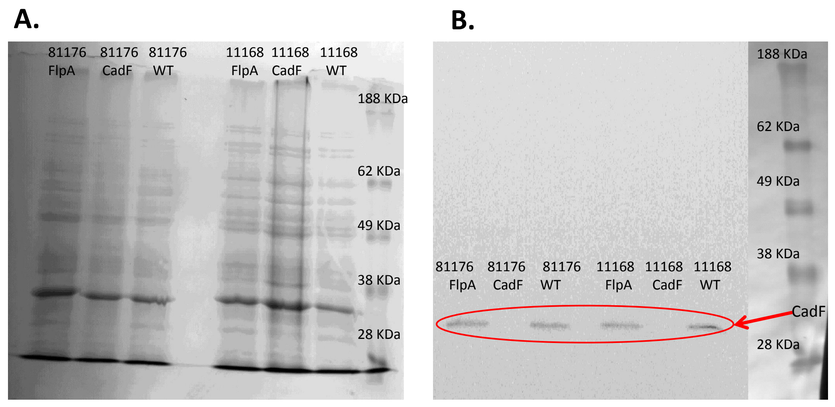


Figure 1. Effects of *cadF* mutation on protein expression. A. Coomassie stain of an SDS-page protein gel showing similar protein expression in all genotypes and serving as a positive control. B. A Western blot of several genotypes of *Campylobacter jejuni* showing the absence of CadF protein in *cadF* mutants in both 11168 and 81176 strains.

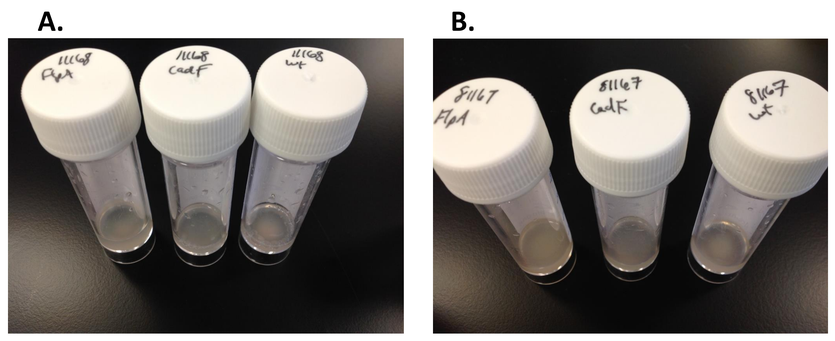


Figure 2. Agglutination assay. A. Agglutination assay of 11168 strain bacteria in PBS. B. Agglutination assay of 81176 in PBS. Changes in optical density from the top layer of PBS over time were used to determine the amount of agglutination. In both figures, clear clumping of bacteria is visible in the right-hand, WT tube. Less agglutination in visible in the adhesin mutants for both strains.

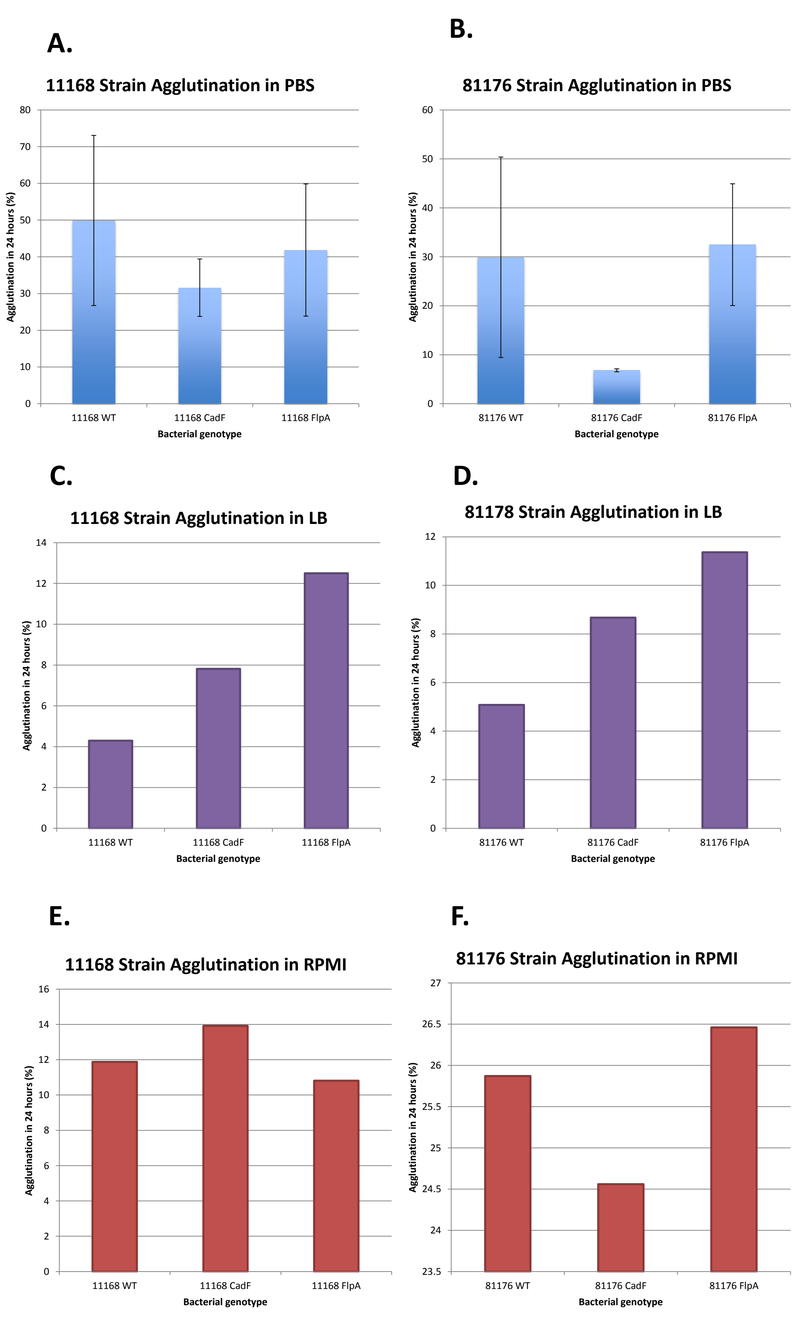


Figure 3. Agglutination assay results. Agglutination experiments were conducted with *C. jejuni* 11168 and 81176 WT bacteria and adhesin mutants in PBS (A, B), LB Broth (C, D) and RPMI media (E, F). Standard deviation for agglutination in PBS is shown. As n=1 for LB and RPMI experiments, no standard deviation was calculated.

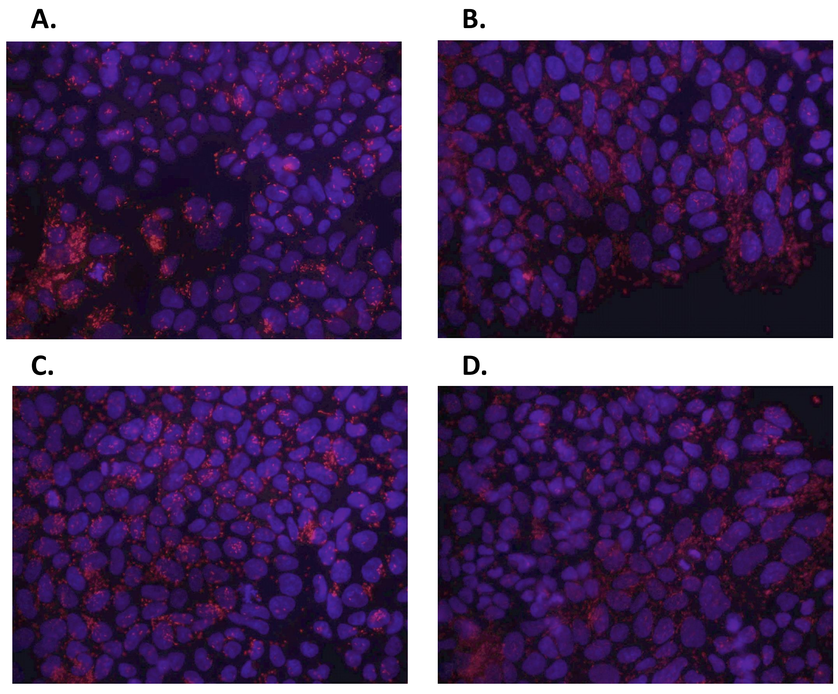


Figure 4. Effects of fibronectin-binding proteins on epithelial cell invasion. Fluorescent microscopy was used to visualize invasion of *Campylobacter jejuni* bacteria. DAPI stained epithelial cell nuclei blue and a *campylobacter*-specific antibody stained bacteria red. Invasion assays were conducted with 11168 and 81176 strains with WT bacteria (A, B), *cadF* mutants (C, D) and *flpA* mutants (E, F).

DISCUSSION

- SDS-page gels, coomassie, and Western blots confirm that *cadF* mutants are lacking in the CadF protein, yet CadF expression in *flpA* mutants is not affected.
- Agglutination occurs at a decreased rate in *cadF* mutants in PBS. This is showing that lack of CadF protein affects the bacteria's ability to self-bind or clump.
- In LB, agglutination may not be related to adhesins. Further replicates are required.
- In RPMI, much less agglutination is occurring in all samples. Perhaps components in the RPMI complete media are preventing adhesin interaction or bacteria binding and clumping to reduce agglutination.
- A reduced number of 81176 *flpA* mutant *C. jejuni* invaded the epithelial cells in comparison to the 11168 strain, as well as *cadF* mutants and WT of 81176.

CONCLUSION

- CadF expression is not altered in *flpA* mutants, as qualified by an SDS-page gel and Western blot.
- The solution in which agglutination assays are conducted appears to have an effect on the agglutination of bacteria. Conducting agglutination experiments in solution which simulates infectious conditions may give more clinically relevant results.
- In *C. jejuni* 81176, FlpA is highly involved in epithelial cell invasion. Future replicates and experiments should be conducted to confirm this conclusion.