Motility related gene expression of *Campylobacter jejuni* NCTC 11168 derived from high viscous media

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Aim

Campylobacter jejuni (*C. jejuni*) has been considered as a major foodborne pathogen. *C. jejuni* is capable of swarming in high viscous surroundings, such as mucus lining the gastrointestinal tract. The aim of this study is to investigate motility related gene expression patterns of *C. jejuni* swarming in high viscous media and liquid culture (low viscous media).

Methods

1 µL of *C. jejuni* NCTC 11168 overnight culture were dropped in the middle of high viscous media made of Brucella broth and 0.4% agarose. Bacterial cells were collected with surrounding media derived from center or edge of bacterial swarming halo formed after incubation at 37° C for 24 h under microaerobic condition (Fig. 1). Further, cells cultured under same conditions in Brucella broth (low viscous) were collected. All samples were centrifuged, the cell pellets lysed by QIAzol and total RNA extracted by chloroform treatment and isopropanol precipitation. Total RNA was treated with DNase I before cDNA was synthesized with random hexamer primers. The expression level of selected genes was determined by RT-qPCR using SsoFast™ EvaGreen Supermix. Primers used were designed in this or previous studies[2,3,4,5,6] and performed with efficiency ranges within 90% - 105%. The relative expression levels were calculated by the $2^{-\Delta\Delta CT}$ method[1] with normalization to the expression level of *rpoA*[7].

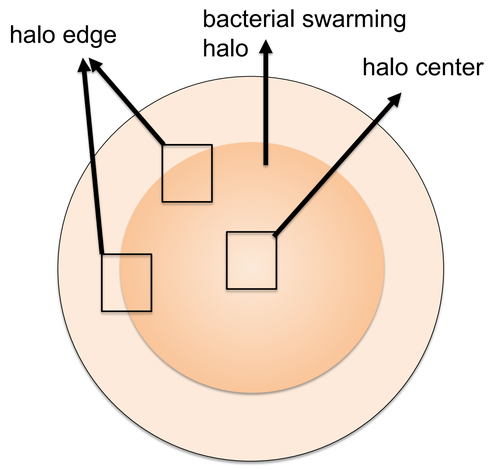

Figure 1: Sample collection points of bacteria swarming in high viscous media

Results and discussion

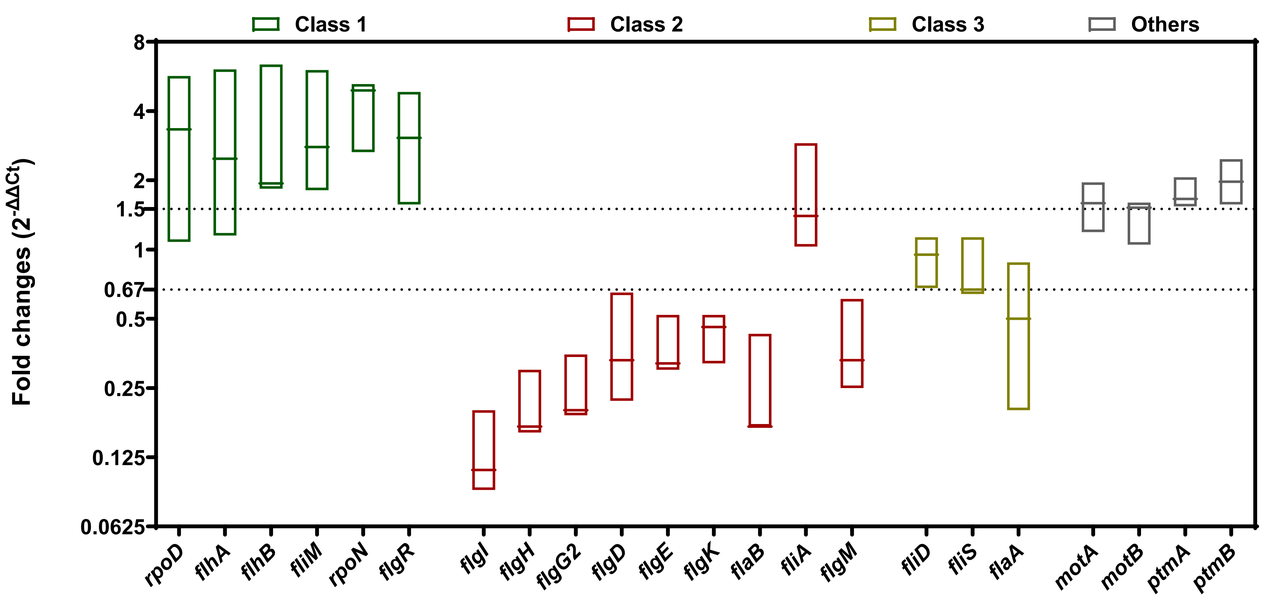

Figure 2: Gene expression pattern of *C. jejuni* swarming in high viscous media.

According to their transcriptional hierarchy, the flagellar genes are assigned to 3 classes. The expression level was analyzed by RT-qPCR using 3 independent cDNA samples with 2 technical replicates in each run. The gene expression profile at the edge of the swarming halo was normalized to the halo center. Min to max floating bar with median of the $2^{-\Delta\Delta Ct}$ fold changes are shown. The dotted lines show the threshold (0.67 to 1.5) for the relevant up and down regulation.

Most of the genes showing higher expression in bacterial cells at the edge of the swarming halo than in the center (Fig. 2) belong to the early flagellar assembly process genes (class 1), including the T3SS components *flhA*, *flhB*, C-ring component *fliM*, and the transcriptional regulators *rpoD*, *rpoN* and *flgR*. Meanwhile, the down-regulated genes belong to the middle flagellar assembly process genes (class 2), including rod associated genes *flgI*, *flgH* and *flgG2*, the hook associated genes *flgD*, *flgE, flgK*, and the minor flagellin *flaB*. Of the genes belonging to the late stage of flagellar assembly (class 3), the major flagellin gene *flaA* was lower expressed, whereas genes encoding filament capping protein (*fliD*) and export chaperon (*fliS*) did not show relevant expression differences. Gene expression for motor stator complex and flagellin glycosylationproteins (others) were slighted enhanced at the edge of halo. These may indicate that the majority of bacteria at the halo edge are likely in an earlier flagellar assembly processing stage than bacteria at the center.

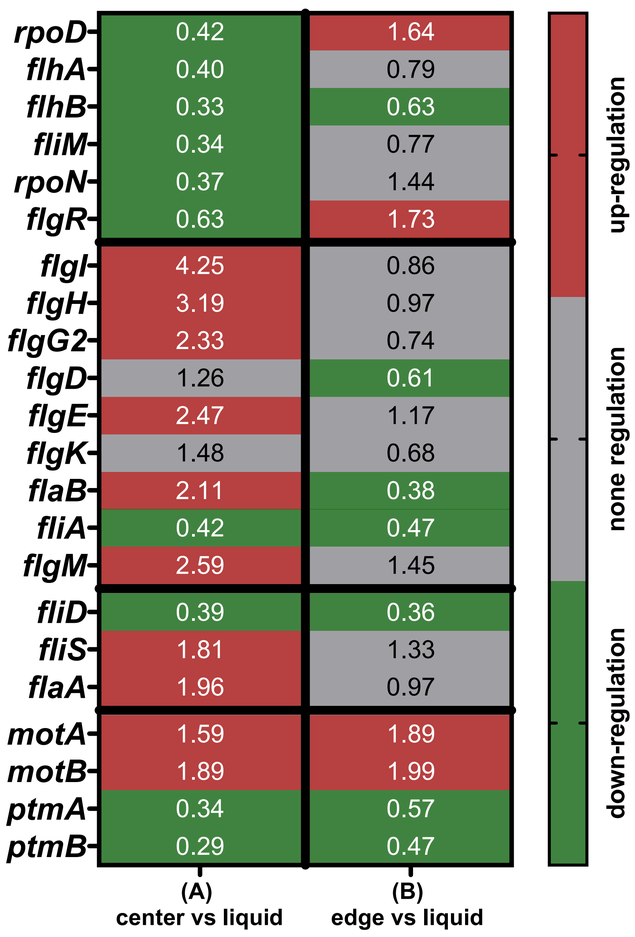

Figure 3: Gene expression of *C. jejuni* swarming in high viscous media was compared to liquid culture. (A) The expression pattern at the halo center compared to liquid culture. (B) The expression pattern at the halo edge compared to liquid culture.

Median of the $2^{-\Delta\Delta Ct}$ fold changes are shown. Non-regulation was defined within the thresholds of 0.67 and 1.5 fold.

In the expression pattern of high viscous media compared to liquid culture (Fig. 3), most genes have shown differential expression in the halo center compared to liquid culture, while less genes were differentially expressed comparing the profiles of the halo edge to liquid cultures. These results suggest that bacteria on halo edge are in a similar flagellar assembly processing stage like the bacteria in the low viscous media after 24 h incubation. However, several genes shared same regulatory direction within center and edge compared to liquid culture, including up-regulated expression of *flhB*, *fliA*, *fliD*, *ptmA* and *ptmB*, as well as down-regulated expression of *motA* and *motB*. This indicates a different pattern of gene expression, possibly related to the viscosity of the surrounding media. However, expression of the genes *rpoD*, *flgR* and *flaB* were regulated in opposite directions.

Summary

Gene expression differences of the flagellar apparatus were observed between bacteria at the edge of the swarming halo and the center in high viscous media and between high viscous media and liquid culture (low viscous media).

Acknowledgement

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Reference

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