Differentiation of the virulence potential of three Campylobacter jejuni strains by use of gene transcription analysis and a Caco-2 assay

Poli, Vanessa Fadanelli Schoenardie; Thorsen, Line; Olesen, Inger; Jespersen, Lene

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DEPARTMENT OF FOOD SCIENCE, FOOD MICROBIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

Differentiation of the virulence potential of three *Campylobacter jejuni* strains by use of gene transcription analysis and a Caco-2 assay

Vanessa F. S. Poli, Line Thorsen, Inger Olesen, and Lene Jespersen

Introduction

Campylobacter jejuni is the leading cause of bacterial diarrhoeal disease in humans. Contaminated poultry and poultry products are recognized as the main vehicle of infection

Recently, environmental conditions have been shown to have a large impact on the virulence of food borne pathogens such as *Listeria monocytogenes* (Olesen et al., 2009). *C. jejuni* is likely to encounter a wide range of temperatures during a contamination cycle. Modulation of *C. jejuni* virulence in response to environmental stresses such as temperature shifts may have further implications in the pathogenesis of campylobacteriosis.

This study investigates the effect of various food related temperature shifts on the survival, and the invasion capacity to the human intestinal epithelial cell line Caco-2, of three different *C. jejunii* strains (two clinical strains and a chicken strain). The effect of the temperature shifts on the transcription of selected virulence and stress genes is also investigated.

Materials and methods

Campylobacter jejuni strains NCTC11168 (clinical, sequenced strain), DFVF1099 (chicken isolate), and TB1048 (clinical strain) were grown in BHI-broth under micro-aerobic conditions at 42℃.

The 16-h cultures obtained at 42℃ (control cells) were shifted to 4℃ for 24 h, and then from 4 to 37℃ for 30 min.

Quantitative real time PCR (qRT PCR) was set up using the TaqMan based chemistry, Applied Biosystems, Inc. Reference genes used were the transcription factor *rpoB,* and *htrB* involved in synthesis of lipid A.

Infection of Caco-2 mono layers with bacterial cells obtained from the various temperature conditions was performed at 37℃ for 3 h.

Results

There was almost no increase or decrease in cell counts under the temperature conditions examined, with the results being highly similar for the three *C. jejuni* strains (results not shown).

The effect of temperature shifts on the transcription of selected virulence and stress genes in the three *C. jejuni* strains is shown in Figure 1.

For strain NCTC11168 (clinical), a statistically significant increase in the relative transcription of the toxin encoding gene *cdtB* and the stress related gene *clpP* was observed at 24 h at 4℃, and when shifting the cells from 4℃ to 37℃ for 30 min (*cdtB* only).

Results continued

1.For strains DFVF1099 (chicken) and TB1048 (clinical), no statistically significant differences in relative transcription levels of *clp* and *cdtB* were observed at any of the temperature conditions examined.

For NTCT11168, DFVF1099 and TB1048 the relative transcription of two genes important for adhesion (*cadF*) and invasion (*ciaB*) was not significantly different after exposure to 4 and 37°C as compared to at 42℃.

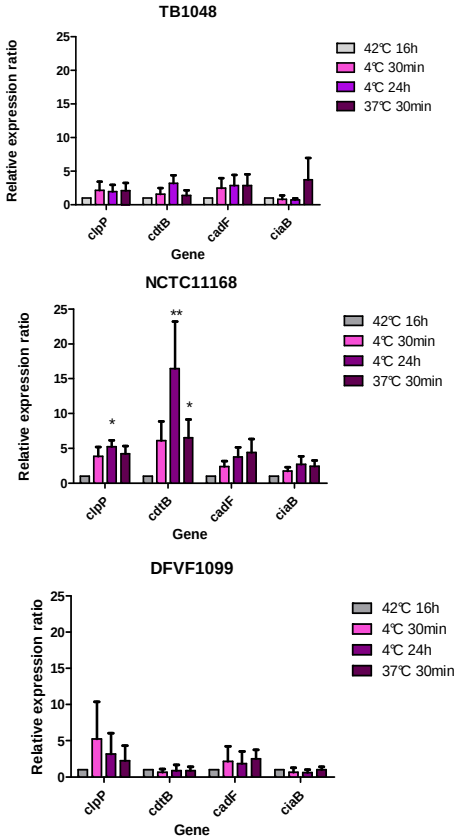

Figure 1. Relative change in the transcription level for three virulence genes (*cdtB, cadF, ciaB*) and one stress response gene (*clpP*) after exposure to temperature shifts. The columns represent mean fold changes, and error bars show standard deviations around the means. The fold change of each stress condition was calculated relatively to the expression levels at the standard condition (42℃ f or 16 h) that was set to 1.0 (P* ≤ 0.05).**

As seen in Figure 2, there was no significant change in the number of adhering and invading *C. jejuni* cells to Caco-2 cells under the temperature conditions examined compared to control cells. However great strain differences were observed, as the chicken isolate was not able to invade the Caco-2 cells, whereas this was the case for the two clinical strains

Reference
Olesen, I., Vogensen, F.K., Jespersen, L., 2009. Gene transcription and virulence potential of *Listeria monocytogenes* strains after exposure to acidic and NaCl stresses. Foodborne Pathogens and Disease 6, 669-679.

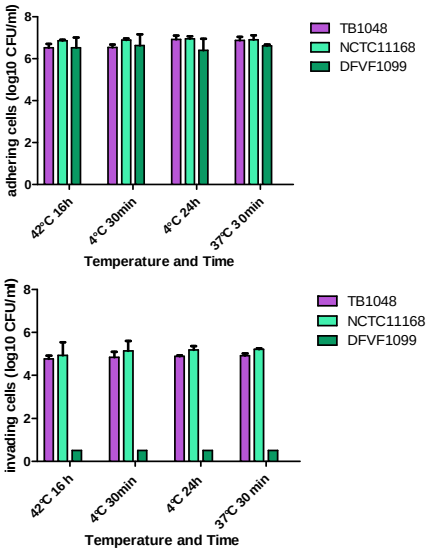

Figure 2. Adhesion and invasion of *C. jejuni* strains to the human intestinal cell line Caco-2 after temperature shifts. The time point used as the control was 42℃, 16 h. The results are presented as $\log_{10}$ of the mean ± SD CFU/ml.

Conclusions

1) The food storage related temperature shifts induced expression of virulence and stress genes in a strain dependent manner. Low temperature storage could increase the expression of virulence genes of certain strains.

2) The temperature shifts did not influence the adhesion/invasion capacities of *C. jejuni.* This was also displayed by the qRT-PCR analysis of the *cadF* and *ciaB* genes, which are known to be involved in the adhesion and invasion process of *C. jejuni*.

3) The three strains, all exhibited the same adhesion capacity to human intestinal cells, while the invasion ability into Caco-2 cells was observed in only the clinical strains. Thus, the clinical strains appeared to be more virulent than the chicken isolate.

4) The qRT-PCR analysis and Caco-2 assay showed to be useful tools for differentiating the virulence potentials of *C. jejuni* strains.

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Contact information
Line Thorsen,
Faculty of Life Sciences
University of Copenhagen
Rolighedsvej 30
DK-1958 Frederiksberg C
Denmark
+45 35 33 33 26
lith@life.ku.dk