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Short communication

Influence of mutation in *cj0183* and *cj0588* genes for colonization abilities of *Campylobacter jejuni* in Caco-2 cells using confocal laser scanning microscope

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Abstract

The *cj0183* and *cj0588* genes identified in the *Campylobacter jejuni* NCTC 11168 genome encode proteins with homology to virulence factors found in other bacteria. Previous studies showed that single mutation in the *cj0183* gene does not affect adhesion of *C. jejuni* to the Caco-2 cell line whereas protein encoded by *cj0588* is involved in adherence to the Caco-2 cells. In the presented study differences in invasion index were observed between mutants in both genes and single mutation of *cj0588* in 81116 and 81-176 *C. jejuni* strains. This fact indicates that Cj0183 protein might play some role in invasion of bacteria into host cells.

Key words: *Campylobacter jejuni*, adhesion, invasion, confocal microscopy

Introduction

Campylobacter jejuni colonizes the intestinal digestive tract of animals, especially birds, as commensal microbiota. Bacterial transmission to humans, inducing severe gut inflammation, occurs mainly due to the improperly prepared poultry products. Studies demonstrated that adhesion and invasion ability of *C. jejuni* promote the process of colonization. Several cell lines of human and non-human origin have been used to characterize the interaction of *C. jejuni* with host cells (Dasti et al. 2010). Caco-2 cells are most commonly used as an assay which is useful to mimic the behavior of *Campylobacter* in both chicken and human gut (Hanel et al. 2004).

Our previous studies indicate that mutation in the *cj0588* gene influences the adherence abilities of *C. jejuni* to the Caco-2 cell line. This mutation reduces both adhesion and internalization of 81-176 and 81116 *C. jejuni* strains to the epithelial cell line (Sałamaszyńska-Guz and Klimuszko 2008). Interaction studies of the purified Cj0588 protein with

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A

Strain	Invasion index %	Reference
81-176	10.14 ± 1.02	(Sałamaszyńska-Guz and Klimuszko 2008)
81-176-ΔCj0588	9.1 ± 0.09	(Sałamaszyńska-Guz and Klimuszko 2008)
81-176-ΔCj0183ΔCj0588	6.04 ± 0.95	this study
81116	17.63 ± 3.1	(Sałamaszyńska-Guz and Klimuszko 2008)
81116-ΔCj0588	11.07 ± 1.76	(Sałamaszyńska-Guz and Klimuszko 2008)
81116-ΔCj0183ΔCj0588	6.92 ± 1.15	this study

B

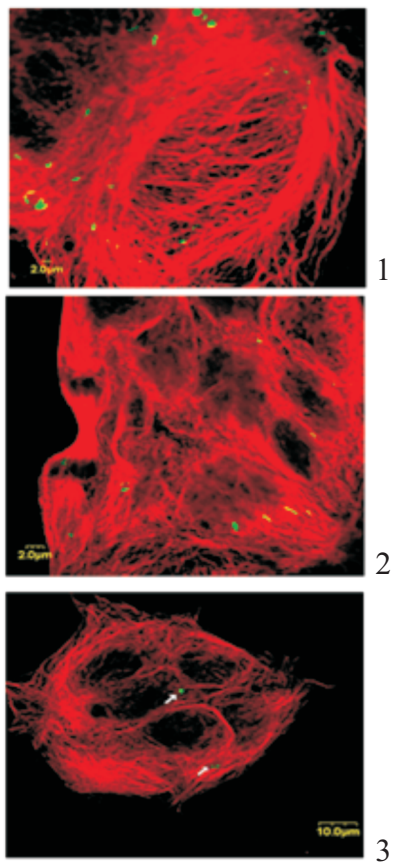

Fig. 1. A) Comparative analysis of invasion index of the wild type 81-176 strain and mutants 81-176-ΔCj0588, 81-176-ΔCj0588Cj0183 and wild type 81116 strain and mutants 81116-ΔCj0588, 81116-ΔCj0183ΔCj0588. Values represent means ± S.E.M. of three independent experiments (P<0.05). B) Representative confocal fluorescence microscopic images of *C. jejuni*-infected Caco-2 cells 4 h after infection. The microtubules (red) appear as structural skeletons outlining the cells and the FITC-labeled bacteria (green) appear as spots along the microtubules. B1) *C. jejuni* 81-176, B2) *C. jejuni* 81-176 ΔCj0588), B3) *C. jejuni* 81-176 ΔCj0183ΔCj0588.

eukaryotic cell proteins were performed what would allow establishing whether the purified protein cj0588 recognizes cellular receptor sites. Results of the experiments showed that the Cj0588 protein binds *in vitro* with the surface of Caco-2 cells (Sałamaszyńska-Guz and Klimuszko 2008). Studies performed using the homologs of the *cj0588* gene – *tlyA* gene, showed that the *tlyA* gene mutation affects colonizing abilities of other pathogenic bacteria – *Brachyspira hyodysenteriae* and *Helicobacter pylori* (Hyatt et al. 1994; Martino et al. 2001).

The aim of the presented work was to determine *in vitro* adherence and an invasion of Caco-2 cells by constructed mutants in both *cj0183* and *cj0588* genes in 81116 and 81-176 strain (81116-ΔCj0588Cj0183 and 81-176-ΔCj0588Cj0183). Detection of internalized *C. jejuni* wild type (81116 and 81-176 strain) and mutants (81116-ΔCj0588, 81-176-ΔCj0588 and 81116-ΔCj 0588Cj0183, 81-176-ΔCj0588Cj0183) in Caco-2 cells was performed using confocal microscopy.

Materials and Methods

Adhesion and invasion assays for *cj0183* and *cj0588* mutants were performed as described by Sałamaszyńska-Guz and Klimuszko (2008). The *C. jejuni* cells and the Caco-2 cell tubulin were visualized using mouse monoclonal anti-bovine -tubulin antibodies, Alexa Fluor 546 conjugated goat anti-mouse IgG (Molecular Probes Inc.) and polyclonal anti – *Campylobacter jejuni* – FITC conjugate (Fitzgerald). Caco-2 cells infected with *C. jejuni* wild type as well as adequate mutated strains were observed using confocal microscopy (Fig. 1). Confocal microscopy was performed with a confocal laser scanning microscope FV-500 (Olympus Polska Sp. z o.o., Poland).

Results and Discussion

The invasion index, which describes the percentage of bacteria which have infected epithelial cells compared to the number of cells that have adhered, calculated for the mutants in both genes of 81-176-ΔCj0588Cj0183 and 81116-ΔCj0588Cj0183 strains decrease in comparison to mutant in *cj0588* gene only ($P<0.05$) (Fig. 1). According the previous results (Sałamaszyńska-Guz and Klimuszko 2008) single mutation in *cj0183* gene was not statistically important for adhesion and internalization of 81-176 and 81116 strains to the epithelial cell line but together with protein encoded by mutated *cj0588* gene unexpectedly reduced invasion abilities of examined strains. It may suggest the possible role of Cj0183 protein in colonization of host epithelial cells by *C. jejuni* which was not so far identified.

Acknowledgements

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