Distribution of virulence genes, adhesion and invasion of *Arcobacter butzleri*

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Introduction

Arcobacter has been assigned as a potential pathogen for human health but so far the virulence mechanisms are still unknown. The aim of this study was to investigate the virulence potential of *A. butzleri.* For that, the distribution of putative virulence genes, induction of apoptosis as well as the adhesive and invasive abilities on different cell lines were analyzed for several *A. butzleri* strains. Furthermore, the genes *ciaB*, *cadF* and *cj1349* of these strains were sequenced to elucidate whether the different adhesive and invasive phenotypes depend on alterations in these proteins.

Material and Methods

Bacterial strains
Altogether, 46 strains of food and environmental origin, five human strains (NRZ *Helicobacter*) as well as the strains *A. butzleri* CCUG 30485 and *Campylobacter* (*C.*) *jejuni* 81-176 were included. All strains were grown in Brucella Broth (Oxoid) or on Mueller Hinton (Oxoid) blood agar (MHB) plates and incubated under microaerobic conditions (5% O_2, 10% CO_2) at 30°C for *A. butzleri* and 37°C for *C. jejuni*.

DNA extraction
Bacterial DNA was extracted by the Chelex method and stored at 4°C until used for PCR.

Detection of putative virulence genes
Putative virulence genes were detected by PCR as described by Karadas et al. (2013).

Comparison of CadF, CiaB and Cj1349 amino acid (aa) Sequences
The complete genes *cj1349*, *ciaB* and *cadF* were sequenced and cds translated with standard genetic code by EditSeq (DNASTAR Lasergene v7). Alignment of the amino acid sequences was performed with Multalin v5.4.1 (Corpet 1988).

HT-29 and HT-29/B6 cell lines
The human colon adenocarcinoma cells HT-29 and HT-29/B6 were grown in RPMI1640 medium with 10% fetal calf serum superior (both Biochrom). Each well of a 24-well plate was seeded with 2x10^5 cells and incubated for 48h (HT-29) or one week (HT-29/B6).

Caco-2 cell line
The human colon adenocarcinoma cells were grown in DMEM medium with 10% fetal calf serum superior, 1% nonessential amino acids (all Biochrom), and 5 µg/ml gentamicin (Roth). Each well of a 24-well plate was seeded with 5x10^4 cells and incubated for 3 weeks.

IPEC-J2 cell line
The porcine small intestinal epithelial cell line were grown in DMEM/F-12/HAM medium (Biochrom) with 5% adult pig serum (Sigma), 1% penicillin/streptomycin, 1% insulin/transferrin/selenium (Gibco), and 5 ng/ml epidermal growth factor (Sigma). Each well of a 24-well plate was seeded with 2,5x10^5 cells and incubated for two weeks.

Adhesion and invasion assay
The assays were performed with 3 human (H) and 3 chicken (C) *A. butzleri* strains and the strain *C. jejuni* 81-176 (CJ). The cells were infected with approx. 1x10^8 bacteria. For the adhesion assay, the infected monolayers were incubated for 1h at 37°C. For the invasion assay, the infected monolayers were incubated for 3h followed by a 2h incubation with 300 µg/ml gentamicin. The total of adherent and invasive bacteria were determined by plating serial dilution of the lysates on MHB agar plates. The adhesion and invasion index was calculated as percentage of the inoculum.

Apoptosis assay
The induction of apoptosis was examined 48h after infection of HT-29/B6 cells by TUNEL-staining and fluorescence microscopy.

Results

Putative virulence gene profile
All investigated *A. butzleri* strains carried the putative virulence genes *mviN*, *cadF*, *cj1349*, *ciaB*, *tlyA* and *pldA* while *iroE*, *hecB*, *irgA* and *hecA* were only present in some of the strains.

Adhesive and invasive ability of *A. butzleri*
The *A. butzleri* strains were tested for their adhesive properties on four different cell lines (Fig.1). The strains were grouped according their virulence gene profile (Fig. 1 E). The strains H2 and C3 showed no or only minimal adhesion on all cell lines investigated. All other strains showed adhesive capabilities on all cell lines except for H1 showing no adherence to the IPEC-J2 cells. Highest adhesion indices were observed for the human cell line Caco-2 and lowest for the porcine cell line IPEC-J2.

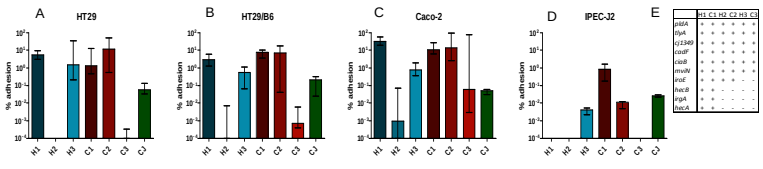

Figure 1: Adhesive capabilities of *A. butzleri* and *C. jejuni* on different cell lines
Adhesion of *A. butzleri* strains and *C. jejuni* 81-176 to the cell lines A) HT-29, B) HT-29/B6, C) Caco-2 and D) IPEC-J2 was detected after 1 h of incubation. Adhesion indices were calculated as percentage of the inoculum. Expressed are the medians ± IQR (n=3). E) Putative virulence gene profiles of the six *A. butzleri* isolates.
H= human strain, C= chicken meat strain, CJ= *C. jejuni* 81-176

The *A. butzleri* strains were tested for their invasive properties in four cell lines (Fig. 2). Only C1 and CJ showed invasive properties on all four cell lines investigated. Even though H3 showed adherence to all cell lines invasive properties could only be observed for the HT-29/B6 and Caco-2 cell lines. The other strains were invasive in the human cell lines but not in the porcine cell line. Highest invasion indices were observed for the human cell line Caco-2 and HT-29/B6 followed by HT-29 and lowest for the porcine cell line IPEC-J2.

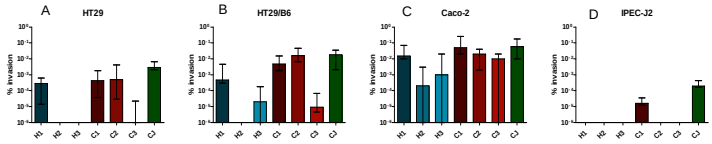

Figure 2: Invasive capabilities of *A. butzleri* and *C. jejuni* 81-176 on different cell lines
Adhesion of *A. butzleri* and *C. jejuni* 81-176 to the cell lines A) HT-29, B) HT-29/B6, C) Caco-2 and D) IPEC-J2 was detected after 3h of incubation followed by a 2h incubation with gentamicin. Invasion indices were calculated as percentage of the inoculum. Expressed are the medians ±IQR (n=3).
H= human strain, C= chicken meat strain, CJ= *C. jejuni* 81-176

Comparison of CadF, CiaB and Cj1349 amino acid (aa) sequences
Even though the aa sequence of Cj1349 (Fig. 3A), CadF (Fig. 3B) and CiaB (Fig. 3C) showed some substitutions the putative functional domains were conserved within all investigated strains. No correlation between adhesive and/ or invasive phenotypes and the amino acid sequence of the three genes could be observed.

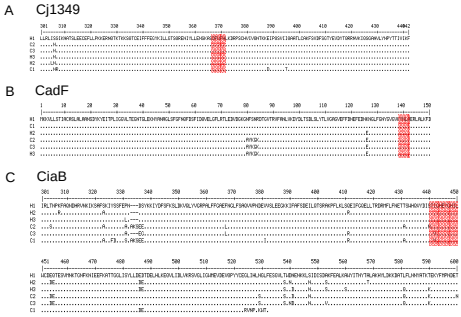

Figure 3: Partial aa sequence alignment of Cj1349, CadF and CiaB
cj1349, *cadF* and *ciaB* were sequenced and cds translated. The aa sequences were aligned and conserved aa indicated as dots. The putative functional domains of Cj1349 (A), CadF (B) and CiaB (C) are highlighted in boxes.

Epithelial apoptosis
Apoptosis in HT-29/B6 cells monolayers induced by all strains were significantly higher compared to control (p< 0.05). Highest apoptotic ratio was induced by strain H2 (Fig. 4A and 4B).

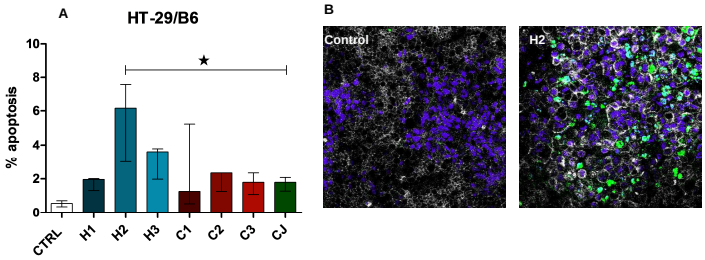

Figure 4: Induction of epithelial apoptosis by *A. butzleri*
Quantification of apoptotic ratio in *A. butzleri*-infected HT-29/B6 monolayers (A) and example of microscopic images of HT-29/B6 cells incubated with *A. butzleri* H2 (B). Apoptotic ratio was calculated as percentage of all cells. Expressed are the mean +- SEM (n=3); (p< 0.05), H= human strain, C= chicken meat strain, CJ= *C. jejuni* 81-176

Summary

All strains carried the putative virulence genes *mviN*, *cadF*, *cj1349*, *ciaB*, *tlyA* and *pldA*. We could demonstrate that *A. butzleri* had *in vitro* virulence potential but the adhesive and invasive properties of *A. butzleri* depend on the cell line and the bacterial strain used. The adhesion and invasion indices of *A. butzleri* for the human colon epithelial cell lines were higher compared to the porcine cell line. Neither the putative virulence gene profile nor the aa sequences of Cj1349, CadF and CiaB could elucidate the different phenotypes observed. The involvement of other genes in virulence as well as the mechanisms should be investigated.

References
Karadas, G., Sharbati, S., Hanel, I., Messelhausser, U., Glocker, E., Alter, T., Golz, G., 2013. Presence of virulence genes, adhesion and invasion of *Arcobacter butzleri*. J Appl Microbiol 115, 583-590.
Corpet, F., 1988. Multiple sequence Alignement with hierarchical clustering. Nucleic acids research 16, 10881-10890.

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