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

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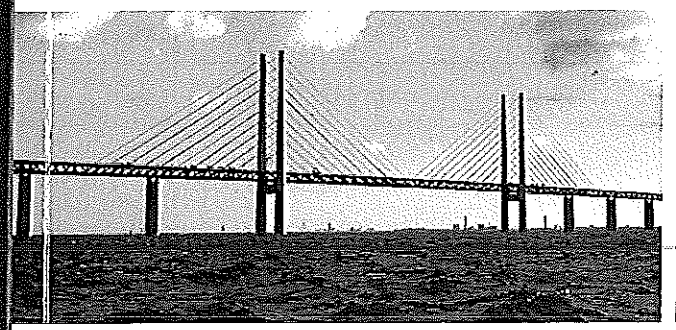

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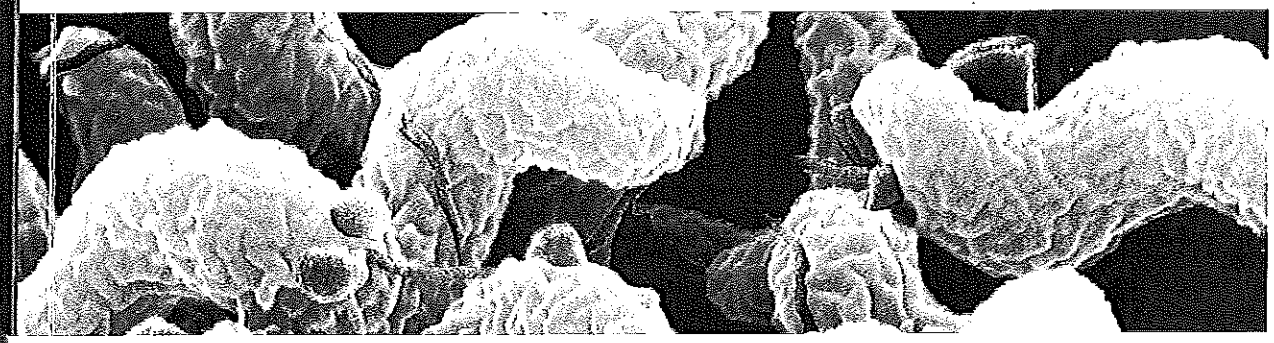

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PEB1.32 Differentiation of the virulence potential of three *Campylobacter jejuni* strains by use of gene expression analyses and a Caco-2 assay

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Campylobacter jejuni is the leading cause of bacterial diarrhoeal disease in humans and has become part of the most important concerns in food safety. Contaminated poultry and poultry products are recognized as the main vehicle of infection. Despite the significance of *C. jejuni* as a foodborne pathogen, little is known about its response to stressful conditions, and, especially, about how it modulates its virulence under such stresses. The aim of the present study was to assess the effect of temperature shift in a broth model system on virulence expression and cell survival of three *Campylobacter jejuni* strains: a clinical isolate (TB1048), a sequenced clinical strain (NCTC11168) and a chicken isolate (DFVF1099). Firstly, cells were transferred from 42 to 4°C to investigate the effect of low temperature storage for short (30 min) and long (24 h) periods of time. Then, the effect of a shift in temperature from 4 to 37°C for 30 min was observed. The virulence properties of *C. jejuni* were evaluated by quantitative Real Time-PCR (qRT-PCR) analysis of the expression of the virulence associated genes *cdtB*, *ciaB*, *cadF* and *clpP*, and by its ability to adhere to and invade Caco-2 cells. The results obtained in this study indicated cell survival and growth inhibition for all strains at 4°C, and no change in cell counts was observed after transfer to 37°C for 30 min. From the virulence perspective, interstrain variation was observed. The expression level of *cdtB* and *clpP* were significantly up-regulated in only one strain (NCTC11168) and invasion ability into Caco-2 cells was observed in the clinical strains only. After exposure to temperature stress, none of the three strains showed significant difference in adhesion and invasion properties as compared to unstressed cells. 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*. As a conclusion, the qRT-PCR analyses and Caco-2 assay showed to be useful tools for differentiating the virulence potentials of the three investigated *C. jejuni* strains under growth conditions were the cell survival was similar. Generally a low storage temperature is not enough to control the survival and virulence of *C. jejuni*.

PEB1.33 The functional importance of *Bacterial lysozyme* Inhibitors

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Lysozymes are ancient and important components of the innate immune system of animals that exert an antibacterial activity by hydrolysing peptidoglycan, the major bacterial cell wall polymer. Three major lysozyme types have been identified in the animal kingdom, commonly designated as the c-type (chicken type), the g-type (goose type) and the i-type (invertebrate type) lysozyme. Although their phylogenetic distribution and expression patterns vary greatly among animals, the defensive role lysozymes against pathogenic bacteria is widely recognised and well documented in different host organisms. From this point of view, it is not surprising that bacteria have in turn evolved mechanisms to evade or subvert the action of lysozyme, f.ex. by producing specific lysozyme inhibitors. Using dedicated function-based screenings for inhibitors of each of the three main animal lysozyme types, we have identified three novel families of lysozyme inhibitors corresponding to each of the three lysozyme families. Although these families of inhibitors lack significant overall similarity at amino acid level, they appear to share a common conserved motif that may be involved in binding to active site residues of lysozyme. This strongly suggests a common ancestral origin for these inhibitors.
Regarding to their function, we demonstrated that knockout of inhibitor production renders bacteria more sensitive to the corresponding lysozyme. Furthermore, challenge experiments using c-type lysozyme inhibitor-defective mutants of APEC (avian pathogenic *Escherichia coli*) in the chicken indicated an important contribution of this lysozyme inhibitor in the virulence of this pathogen. Interestingly, we have strong indications that the g-type lysozyme inhibitor also inhibits a bacterial autolysin that is related to g-type lysozyme, suggesting a role for this inhibitor family in the regulation of autolysin activity. In conclusion, these newly identified lysozyme inhibitors occur in a wide range of gramnegative bacteria, probably originate from a common ancestor, and may have different functions including defence against animal host lysozyme and regulation of autolysin activity. The study of lysozyme inhibitors will provide new insights in bacterial physiology and ecology, and they may constitute an attractive novel target for antibacterial drug development.