Antiadhäsive Naturstoffe gegen *Campylobacter jejuni*

Summary

Infections caused by bacterial species of the genus *Campylobacter* are one of the major causes of severe diarrhoeal enteritis worldwide. Since about 550 million people worldwide suffer from campylobacteriosis and the mortality rate is about 33 million p.a., multifactorial prevention strategies are necassary to reduce the prevalence of *Campylobacter*. In particular, anti-adhesive strategies with specific inhibitors seem to be a promising approach to reduce the bacterial load of *Campylobacter* in poultry production by preventing the initial interaction between host and pathogen. Natural compounds and plant extracts seem to be promising to control *Campylobacter* infections, especially in developing countries. Therefore, natural products that have been reported to be active against *Campylobacter* were investigated for their potential properties against *C. jejuni* in order to develop new strategies for infection prevention.

An *in vitro* flow cytometric adhesion assay was established to study the influence of selected natural compounds on the adhesion of *C. jejuni* to Caco-2 cells. In particular, polysaccharides (e. g. mannans, fructosans), extracts from *D. carota* and *Capsicum sp*. as well as chitosan were identified as antiadhesive compounds and their influence on *C. jejuni* was further investigated in detail. Chitosans exhibit multiple structural variations with regard to the degree of acetylation (DA) and molecular weight (MW). Investigations on the structure-activity relationship of *C. jejuni* adhesion to host cells were conducted. Chitosan 134 (MW 289 kDa, DA 1 %) and chitosan 90/20 (MW 79 kDa, DA 8 %), which are low acetylated and high molecular weight chitosans, appeared to be particularly effective. Both exhibited a highly significant reduction of *C. jejuni* adhesion to Caco-2 host cells (chitosan 134: 64 ± 23 % inhibition; chitosan 90/20: 52 ± 5 % inhibiton, 100 µg/mL respectively).

Subsequently, the anti-adhesive effect of chitosans was verified by epifluorescence microscopy. Chitosans 134 and 90/20 indicated a strong anti-adhesive effect (1 mg/mL and 0,1 mg/mL). Confocal laser scanning microscopy was utilised to investigate the effect of selected chitosans on the invasion of *C. jejuni* into Caco-2 cells. Bacterial invasion was reduced after pre-incubation with chitosan (up to 7% invasion, 1000 µg/mL chitosan 134), probably as a result of the reduction in bacterial adhesion.

The data obtained by the adhesion assay were further investigated by a *sandwich-like in-house* ELISA with recombinant *C. jejuni* adhesins FlpA and JlpA to elucidate binding affinity of chitosan 134 to them. Chitosan 134 showed inhibitoric effects on JlpA in the presence of its natural ligand HSP90a with an EC_{50} = 2.95 ± 5.68 µg/mL, while there was no effect on the binding of FlpA to its ligand fibronectin. This indicates a specific interaction between chitosan and JlpA.

To elucidate the influence of selected chitosans on C. *jejuni* further target identification studies were carried out. The cell integrity of *C. jejuni* was examined by atomic force microscopy, which revealed cell lysis by chitosan. This observation was confirmed by propidium iodide assay, which showed an effect of the higher acetylated and low molecular weight chitosan 661 (MW 19 kDa, DA 20 %) and chitosan 70/20 (MW 64 kDa, DA 23 %) on the membrane integrity of *C. jejuni*. After an incubation

period of up to 48 hours, an adaptation of *C. jejuni* to the chitosans was observed: The membrane integrity of the pre-incubated bacteria showed reduced values in comparison to the untreated control (chitosan 661: 3300 % increase after 2 h and 377 % increase after 48 h incubation time; chitosan 70/20: 4300 % increase after 2 h and 207 % increase after 48 h incubation time, 1 mg/mL respectively; untreated control: 100 %). It is reasonable to assume that *C. jejuni* has a mechanism to counteract the effect of chitosan.

Subsequently, 2D gel electrophoresis and LC-MS were used to study the proteome of *C. jejuni* after 24 h incubation with chitosan 134. Compared to the untreated control group, the treated control group showed a strong influence of chitosan 134 on protein expression. In particular, proteins involved in the stress response, carbohydrate and amino acid catabolism, as well as in cell wall and membrane biosynthesis were differentially expressed. This could have a direct impact on the pathogenicity of *C. jejuni* and the colonisation in host cells.

To simulate real-life conditions, raw chicken meat was treated with chitosan 134 and the number of colony forming units (CFU) of *C. jejuni* was determined. A strong reduction of CFU was observed with a concentration of 10 mg/mL.

In conclusion, the data obtained from these studies show very promising results in reducing *C. jejuni* load *in vitro* and *ex vivo* with low acetylated and high molecular weight chitosans. Chitosans may have high and easy applicability as dietary supplements.

Daucus carota (carrot) was previously reported to be used in diarrhea. Therefore, extracts of *D. carota* were further investigated for anti-adhesive properties regarding *C. jejuni* adhesion to host cells. Crude aqueous extracts (decoction) showed anti-adhesiv effects (64 ± 7 % inhibition, 1 mg/mL). As confirmed by epifluorescence microscopy, the anti-adhesive properties of *D. carota* extract could be attributed to a polysaccharide fraction rich in acetylated rhamnogalacturonan-I (10 mg/ml: 78 % inhibition; 1 mg/mL: 56 % inhibition).