Summary

The antiadhesive effect of an aqueous extract of immature fruits of *Abelmoschus esculentus* (L.). Moench, also known as Okra or Lady's finger, against the adherence of *Helicobacter pylori* on gastric epithelial cells has been proven recently by an *in situ* [Lengsfeld et al., 2004a] and an *in vitro* assay [Messing et al., 2014a]. The antiadhesive activity could not be related to a single polymer, but only to the unpurified extract. Therefore, the aim of this study was to isolate distinct compounds from a freshly prepared water extract of immature Okra fruits with antiadhesive effects against the bacterial attachment. Moreover, the detailed structural features of these polymers had to be correlated to the respective molecular targets of the bacterial surface.

An aqueous extract of pulp material obtained from fresh Okra fruits (FE) was purified by ammonium sulfate precipitation resulting in three high molecular weight subfractions. In a second approach the fresh pulp material was subjected to a stepwise extraction protocol [Sengkhamparn et al., 2009a]. Prior to the extraction of cell wall associated polysaccharides low molecular weight and lipophilic compounds, respectively, were removed. The antiadhesive activity of the several fractions obtained by these extraction procedures was analyzed in an *in vitro* flow cytometric adhesion assay using human gastric epithelial (AGS) cells and different strains of *H. pylori*.

The fractions $FE_{60\%}$ and $FE_{90\%}$ (inhibition of 69 % ± 15 % and 75 % ± 11 %, respectively, at 1 mg/mL) obtained by ammonium sulfate precipitation as well as HBSS and CASS (inhibition of 69 % ± 17 % and 53 % ± 8 %, respectively, at 1 mg/mL) originating from the stepwise extraction protocol exhibited the highest antiadhesive activity and were therefore selected for a detailed chemical characterization. These inhibitory effects were shown not to be strain-specific. For the structural elucidation analysis of the molecular weight, the carbohydrate composition as well as the D-/L-configuration of the respective monosaccharides, and linkage analysis was performed. The main structural features in the fractions $FE_{60\%}$, $FE_{90\%}$, and HBSS were identified as rhamnogalacturonan I (RG I) backbones bearing short side chains of one to three galactose moieties. This structure was confirmed by 1D- and 2D-NMR analysis. By selective enzymatic cleavage the side chain composition was determined in detail. The polysaccharides present in the CASS fraction were identified as xyloglucans. The detailed structure was elucidated after enzymatic cleavage of the polysaccharide backbone with subsequent analysis of the released oligosaccharides by ESI-Q-TOF-MS/MS.

The esterification of the pectic polymers was shown to be a prerequisite for the antiadhesive activity, since the saponified polymers exhibited a strongly reduced effect. Saponified $FE_{60\%}$ and $FE_{90\%}$ reduced the bacterial binding to 11 % ± 15 % and 11 % ± 26 %, respectively, at 1 mg/mL, while saponified HBSS exhibited an inhibition rate of 52 % ± 24 % at the same concentration. Comparing the antiadhesive activity of standard pectins with varying degree of esterification (DE) and of different sources, a higher DE resulted in stronger inhibitory effects, whereas the degree of acetylation presumably has a higher impact than the degree of methylation. Thus high molecular weight polysaccharides exhibiting a high amount of rhamnogalacturonan I regions as well as a high degree of acetylation were assessed as a typical structural requirement for the antiadhesive activity against the BabA- and SabA-mediated adhesion of *H. pylori* to the host cells.

By dot blot overlay assay the isolated pectic Okra polysaccharides were shown not only to interact with the major *H. pylori* adhesins BabA and SabA, but also with yet unknown receptors on the bacterial surface. Thus, the polymers $FE_{60\%}$ and $FE_{90\%}$ were identified as the main active compounds exhibiting the antiadhesive activity.