DNase treatment to reduce *Campylobacter* - and *Pseudomonas*-biofilms

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ONE HEALTH APPROACH
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Aim

Campylobacter (*C*.) has the ability to survive outside the host, presumably in the viable but not culturable (VBNC) state or in biofilms. They are able to colonize and survive in the microaerobic milieu of existing biofilms, containing e.g. *Pseudomonas* (*P*.) spp., which might lead to a continuous entry into poultry houses, contaminations during food processing or directly to human infections from environmental sources. In biofilms, microorganisms are surrounded by so-called extracellular polymeric substances (EPS), which are generally composed of nucleic acids, proteins and polysaccharides. This EPS structure protects the microorganisms from adverse external circumstances and makes the elimination of the microorganisms by common cleaning measures more difficult. The reduction of biofilms with DNases offers a potential application to reduce the risk of cross-contamination.

Methods

Bacterial Strains Experiments were performed using the strains *C*. *jejuni* 81-176 + pMW1007-*gfp* vector (kindly provided by Steffen Backert, FAU) [1], *C*. *jejuni* F38011 (kindly provided by Xiaonan Lu, UBC), and *P*. *aeruginosa* DSM 1117.

Biofilm assay Single species biofilms as well as mixed biofilms of both species were grown in sterile 96 well polystyrene micro-titer plates in Mueller-Hinton broth for 72 h at 37 ºC under microaerobic conditions. Afterwards the biofilm mass was determined by crystal violet staining and measurement of the absorbance.

DNase treatment After removing the supernatant of the maturated biofilms three different DNase I (2 U/ml) treatments were investigated (Fig. 1).

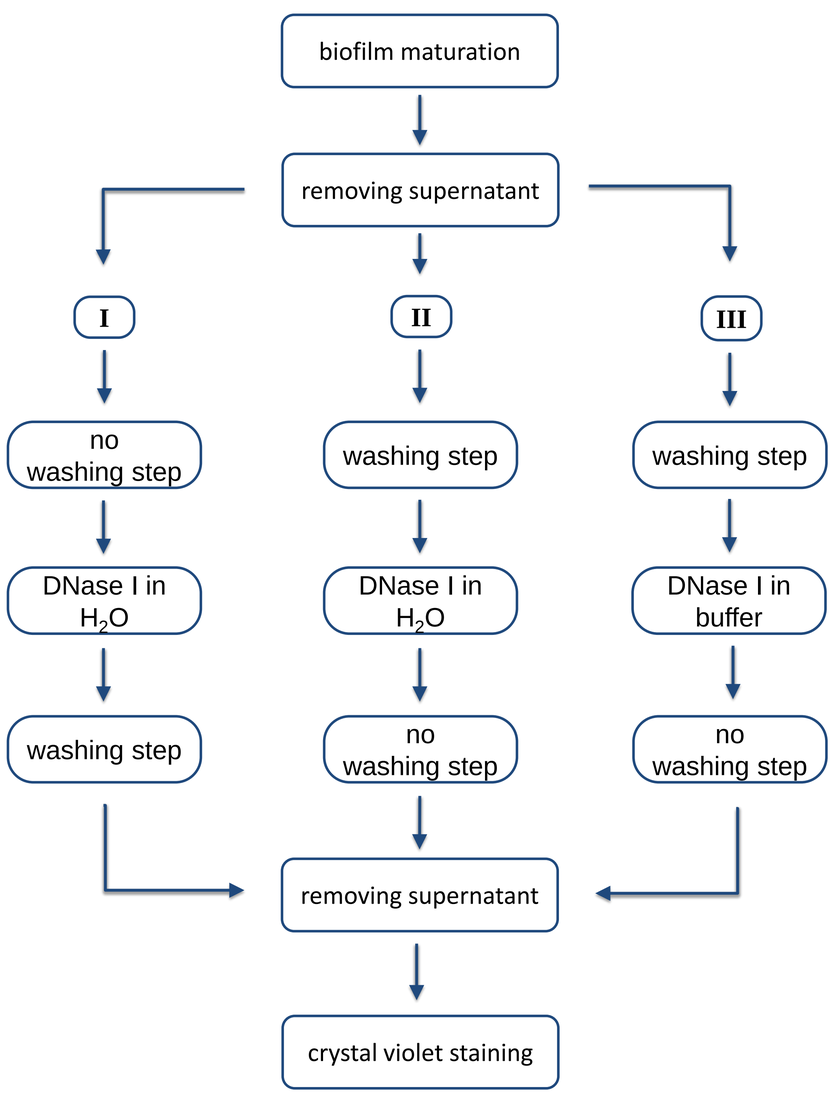

Figure 1: Scheme of DNase I treatments

Results

Overall *C*. *jejuni* formed significantly less biofilm mass compared to *P*. *aeruginosa*. Further the dual-species biofilms showed no further increase in the biofilm mass compared to *P*. *aeruginosa* mono-species biofilms. A significant reduction up to 80 – 90 % for *C*. *jejuni* biofilms and 40 – 60 % for *P*. *aeruginosa* dual-species biofilms were determined after treatment of maturated biofilms with DNase I in water, without a previous washing step (I) (Fig. 1).

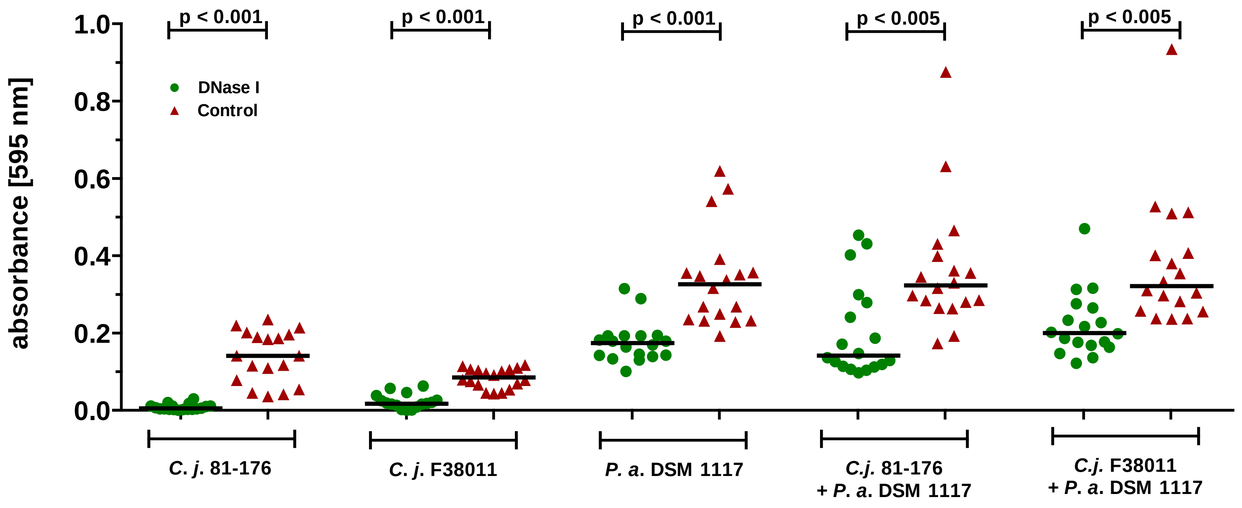

Figure 2: Measurement of the absorbance from maturated biofilms with and without DNase I treatment.
The DNase I treatment was performed in water without a previous washing step (I), for mono- and dual species biofilms. Shown are the results of three independent experiments with the median. Statistical analysis was done using a two-sided Mann-Whitney U test and 95 % confidence interval.

If the maturated biofilms were washed before the treatment with DNase I in water (II), no reduction in the biofilm mass of *P*. *aeruginosa* and the dual-species biofilms was observable in contrast to *C*. *jejuni*. However, if the washed biofilms were treated with DNase I in an appropriate buffer containing $MgCl_2$ (III), a reduction for the *P*. *aeruginosa* and dual-species biofilms was observable (Fig. 2).

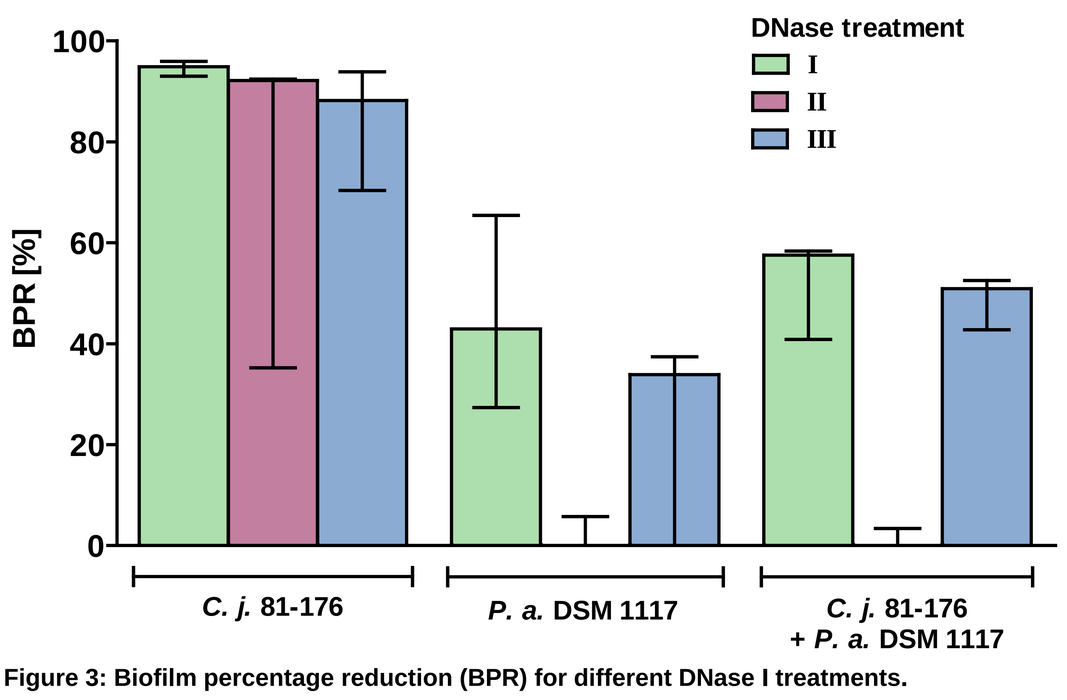

Figure 3: Biofilm percentage reduction (BPR) for different DNase I treatments.
Shown are the calculated percentage reductions of three independent experiments with the median ± upper and lower limit.

Conclusion

These data indicate that the effectiveness of reduction strategies is considerable influenced by the microbiological composition of the biofilms and thus also the composition of the EPS structure.

Further, despite the knowledge about the bacterial composition of existing biofilms along the food chain, it is important to use defined conditions for the DNase I treatment to efficiently reduce the existing biofilms.

Acknowledgement

This study received financial support from German Federal Ministry for Education and Research (PAC-Campy, Grant reference 01Kl1725A).

Federal Ministry of Education and Research

Reference

1. Krause-Gruszczynska, M., et al.,. Cell Microbiol, 2007. **9**(10): p. 2431-44.