Identification of multi-species biofilms in the meat processing environment and characterisation of involved bacteria in a mono- and multi-species biofilm model

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Biofilms are suggested to be a source of contamination in the food producing environment leading to food spoilage or the transmission of food-borne pathogens. However, to date, research has mainly focused on the presence of (biofilm-forming) bacteria within food processing environments, without analysing the associated biofilm matrix components.

The aim of this study was to identify biofilm hotspots in a meat processing environment by analysing the presence of microorganisms (by cultivation and targeted quantitative real-time PCR based on 16S rRNA) and the major matrix components carbohydrates, extracellular DNA and proteins. Sampling included 47 distinct food contact surfaces and 61 distinct non-food contact surfaces from eleven rooms within an Austrian meat processing plant, either during operation or after cleaning and disinfection. Additionally, we isolated and characterized bacteria found in biofilms. The biofilm forming capacity of eleven isolates, was tested, using a static biofilm model. Additionally, two different multi-species settings were tested combining three strains, each. Biofilms were grown on stainless-steel slides for seven days at 10 °C, to mimic conditions found in the food producing environment.

Overall, we identified ten biofilm positive sites, among them seven of which were sampled during operation and three after cleaning and disinfection. Five biofilms were detected on food contact surfaces (cutters and associated equipment and a screw conveyor) and five on non-food contact surfaces (drains and water hoses) resulting in 9.3 % of the sites being classified as biofilm positive. From these sites we cultivated bacteria of 29 different genera. The most prevalent bacteria belonged to the genera Brochothrix, Pseudomonas and Psychrobacter. From each biofilm we isolated bacteria from four to 12 different genera, indicating the presence of multi-species biofilms.

Culturing of eleven isolates of different species (all detected in the mentioned biofilms, representing typical residential and spoilage bacteria in the meat processing environment) showed that there are differences of individual strains to produce matrix components and biomass on stainless steel slides. Brochothrix, Carnobacterium and Kocuria produced only detectable amounts of carbohydrates but neither eDNA nor proteins. The Acinetobacter and the Flavobacterium isolates were able to produce two of the measured components and six strains were capable of producing all types of analysed matrix components, among them a Pseudomonas fragi isolate. The minimal mean
The bacterial load detected was 5.4 log CFU/cm² formed by the Psychrobacter strain.

Different isolates showed differences in matrix formation ability, possible contributing in different amounts to the matrix production in multi-species biofilms, indicating that multi-species biofilms are a key survival mechanism for microorganisms within the food processing environment.

Currently, we are testing two different multi-species biofilms in our model. Hereby we cultivate three species detected in the cutter-associated biofilms and other three species detected in the water hose-associated biofilms together to mimic these biofilms. This work ultimately showed the presence of multi-species biofilms within the meat processing environment, thereby identifying various sources of potential contamination. Data on the presence, formation and composition of biofilms (i.e. chemical and microbiological) will help to prevent and reduce biofilm formation within food processing environments.