Survival of *Salmonella enterica* and *E. coli* O157:H7 in environmental biofilms isolated from pork plants as compared to beef plants

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Background

S. enterica and *E. coli* O157:H7 are leading causes of foodborne illness in the U.S. Biofilm formation by these pathogens may play an important role in meat contamination. Previous studies suggested that certain *S. enterica* and *E. coli* O157:H7 strains may be better at adapting to the processing environment with stronger survival ability via mixed biofilm formation with natural microbial communities and subsequently, a higher chance of causing contamination. Our recent study using natural multispecies biofilms recovered from a processing plant experiencing an increased O157 prevalence showed these processing plant environmental biofilms recruited and protected *E. coli* O157:H7 to a significantly greater extent than the natural biofilms recovered from a control plant not experiencing similar problems, implying that the plant environment microbial communities may pose the potential to enhance pathogen survival and persistence. Current sanitization studies mostly focus on single-species pathogen biofilms but do not take into consideration that pathogens can be harbored in natural mixed biofilms which are commonly seen in commercial plants. Sanitizer effectiveness can vary significantly due to the interactions between the processing plant environmental microorganisms and the pathogens. Furthermore, in addition to biofilm cell inactivation, post-sanitization pathogen survival control and prevalence prevention are all essential for reducing biofilm–related cross-contamination. Here we evaluated the effectiveness of novel sanitizer products against pathogens harbored in natural mixed biofilms isolated from pork and beef plants, and further examined the impact of the microbial communities present in the different processing environments on pathogen survival and post-sanitization prevalence.

Method & Methods

Two pork plants and three beef plants were selected for environmental sample collection. Floor drains located at areas of the fabrication room and cooler of the pork plants and processing floor, cooler, or hotbox of the beef plants were sampled. Biofilm formation by these samples was determined on 96-well polystyrene plates using the crystal violet staining method. To evaluate sanitizer effectiveness against *S. enterica* or *E. coli* O157:H7 harbored within environmental mixed biofilms, an *S. enterica* or *E. coli* O157 cocktail was prepared by mixing equal volumes of overnight cultures of five *S. enterica* strains of various common serovars (Anatum, Dublin, Montevideo, Newport, Typhimurium) or five *E. coli* O157:H7 strains that were all isolated from trim samples at commercial plants. The floor drain samples were enriched and allowed to form mixed biofilms on stainless steel (SS) or tile chips with the co-inoculation of the *S. enterica* or *E. coli* O157:H7 cocktail under processing temperature (7^{o}C). A multi-component sanitizer and an enzyme-based detergent were tested against the pathogens colonized in mixed biofilms following the manufacturers' instructions using foam coverage or fog treatment. The ability of the mixed biofilms to recruit and/or protect from the treatments' co-inoculated pathogen cells was determined. Further, biofilm morphology before and after treatment with the enzyme-based detergent was directly visualized using a scanning electron

microscope (SEM) to better understand its functional mechanism. DNA extraction and 16S rRNA gene amplicon metagenomic analysis was performed to analyze and compare the microbial communities collected from the pork and the beef plants to determine if the environmental community compositions as the result of the different processing operations are related to the observed phenotypic difference.

Results

All pork plant samples formed strong biofilms whereas variations in the biofilm-forming ability of the beef plant samples were observed, which depended upon the plants and drain locations. *S. enterica* and *E. coli* O157:H7 strains were able to integrate into all mixed biofilms efficiently, even under relevant low temperatures (7^{o}C). The multi-component sanitizer applied as foam coverage reduced environmental bacteria and pathogen cells in most samples to a non-enumerable level. However, higher survival of environmental biofilm cells was observed on the tile surface as compared to the SS surface, suggesting contact surface materials and texture would affect biofilm stress tolerance. After overnight enrichment, a higher post-sanitization prevalence of both pathogens was observed in the treated pork plant biofilms than those colonized in beef plant biofilms. Fog treatment was less effective than foam coverage as more biofilm samples had viable/enumerable bacterial cells after the treatment. Fog treatment reduced *E. coli* O157:H7 to a non-detectable level and prevented its post-treatment recovery in all samples, however, the colonized *S. enterica* strains exhibited higher tolerance as enumerable *Salmonella* cells were observed in all pork plant biofilms and in 30% of beef plant biofilms. SEM analysis showed that the enzyme-based detergent dissolved the mixed biofilm extracellular connection and altered the biofilm morphology. No intact mature biofilm architecture was found after treatment, instead, scattered clusters of bacterial aggregates or individual cells were observed which was consistent with the viable bacterial enumeration data. Following the manufacturer's 2-step protocol, quaternary ammonium chloride (QAC) or peroxyacetic acid (PAA) was applied as the second step treatment following the detergent usage, which further reduced viable bacteria in most samples to a non-enumerable level. Again, an overall higher pathogen survival in pork plant biofilms than those in beef plant biofilms was observed after treatments by detergent alone, QAC, PAA, or the consecutive 2-step treatments. The 1st step of detergent treatment reduced pathogen post-sanitization prevalence compared to QAC or PAA treatment alone. PAA used as the 2nd step treatment inhibited *Salmonella* post-sanitization prevalence more efficiently than QAC. However, such higher efficiency of prevalence prevention by PAA was mostly observed while the *Salmonella* strains colonized within beef plant biofilms but not so much while the pathogen was integrated into the pork plant biofilms. The 16S rRNA gene amplicon metagenomic analysis showed differences in microbial compositions of the biofilm samples collected from the pork and beef plants that may be associated with the various phenotypes of the communities present in each processing environment.

Conclusion

The unique natural microbial community composition at the pork plants and the resulting biofilm formation and species interactions might affect the tolerance level of the pathogens integrated into the environmental biofilms and subsequently the effectiveness of sanitization with regards to pathogen inactivation and prevalence prevention. Therefore, research reports on sanitization processes described for beef plants may not apply to pork plants.