

## SANITATION

### REMOVAL AND DISINFECTION OF *LISTERIA MONOCYTOGENES* AND POULTRY SOIL-CONTAINING BIOFILMS USING CHEMICAL CLEANING AND SANITIZING AGENTS UNDER STATIC CONDITIONS (J. F. Frank, J. Ehlers, and L. Wicker)

Cleaning and sanitizing the food processing environment often involves the application of chemical agents in the form of foam or gel (viscous liquid or thin film) to avoid the use of high pressure sprays and hand scrubbing that can facilitate the spread of pathogenic bacteria. In addition, these chemical agents are often applied without application of heat to ambient or cold surfaces. The objective of this research was to evaluate the effectiveness of cleaning and sanitizing chemicals applied under static conditions without application of heat for the removal of *Listeria monocytogenes* biofilms coated with soil of poultry origin. Chemicals evaluated were alkali and neutral cleaning compounds, sodium hypochlorite, acidified sodium chlorite, peroxyacetic acid, peroxyacetic acid/octanoic acid mixture, and quaternary ammonium compound sanitizing agents. Biofilms were prepared by growing *L. monocytogenes* on stainless steel for 24 h at 25°C. The resulting biofilms were then coated with chicken serum albumin and rendered chicken fat. Chemical treatments were at 4°C or 25°C for 1 to 30 min. At 25°C, the alkali cleaning agent removed 99% of fat and 93% of protein after 30 min exposure. The neutral cleaning agent was equally effective at removing fat, but removed only 77% of protein. The alkali cleaning agent also effectively removed *L. monocytogenes* biofilm coated with protein, decreasing cell numbers on the surface by over 7 log<sub>10</sub> after 10 min exposure. Acidified sodium chlorite and peracetic acid/octanoic acid mixture were the most effective sanitizers at killing *L. monocytogenes* biofilm coated with fat and protein, both achieving > 5 log<sub>10</sub> reduction after 1 min exposure at 25°C. A combination of 10 min cleaning with alkali and 30 min sanitizing with acidified sodium chlorite achieved > 7 log<sub>10</sub> reduction of *L. monocytogenes* to nearly undetectable levels (> 0.2 cfu/50 cm<sup>2</sup>) at 25°C. The combination of alkali cleaning (10 min) and use of either acidified sodium chlorite or peracetic acid/octanoic acid (10 min) were effective at inactivating the *L. monocytogenes* biofilm at 4°C, achieving > 6.0 and 5.3 log<sub>10</sub> reductions, respectively. This research has demonstrated that processing plant environmental surfaces can be effectively cleaned and sanitized using static application of chemicals on surfaces and ambient and cold temperatures.

### HEAT INACTIVATION OF *LISTERIA MONOCYTOGENES*-CONTAINING BIOFILMS (R. Chmielewski and J. F. Frank)

Recent outbreaks of *Listeria monocytogenes* have been associated with the consumption of contaminated processed meat products and refrigerated products and have raised concern that recontamination is occurring during or after processing. Possible sources of recontamination in food processing plants could be due to ineffective cleaning and redeposition of soil especially in stagnant areas such as joints and dead ends. The objective of this research was to develop a predictive model to determine the importance of time and temperature for predicting survival of *Listeria monocytogenes*, *Pseudomonas*, and *Listeria*-*Pseudomonas* mixed culture biofilms formed on stainless steel and buna-N rubber coupon surfaces.

Coupons were added to 10% TSB inoculated with 0.1% *Pseudomonas* spp M21, *L. monocytogenes*, or 1:4 *Pseudomonas* spp M21- *L. monocytogenes* mixed culture and incubated for 4 h at 25°C. After attachment, coupon surfaces were rinsed with phosphate buffer and transferred to 10% TSB and incubated for 48 h at 25°C. Duplicate coupons were tested for each heating time (1, 3, 5, or 15 min) and temperature (70, 72, 75, or 77°C). Heat treated samples were enumerated using the fraction negative enumeration method. Positive controls were vortexed with glass beads and enumerated using PCA and *Listeria* selective agar. The experiment was repeated six times.

Time was the predominant predictive factor for biofilm survival on stainless steel while both temperature and time contributed equally to predicting the survival of biofilm on buna-N rubber. Overall, *Pseudomonas* was more heat resistant than *Listeria* on stainless steel, probably due to its higher initial load. On rubber, *Listeria* in the mixed culture biofilm had the greatest probability of survival. *Pseudomonas* in biofilms on stainless steel has a 16% probability of survival after heat treatment of 77°C for 15 min and 0.04% on buna-N rubber. For *Listeria* in

biofilms, the probability of survival is 7% on stainless steel and 0.094% on buna-N while in mixed culture biofilms, the probability of survival of *Listeria* was 0.3% on stainless steel and 0.4% on buna-N rubber.

### **FATE OF AEROSOLIZED *LISTERIA MONOCYTOGENES* IN A CLOSED BIOAEROSOL CHAMBER (Z. Yan, C. M. Lin, J. Kornacki, and M. Doyle)**

The role of aerosols in transmission of *Listeria monocytogenes* in a ready-to-eat meat processing facility environment is uncertain. A 315-liter-enclosed Plexiglas bioaerosol chamber was designed to study the fate of aerosolized *L. monocytogenes* at various levels under selected moisture conditions. *L. monocytogenes* cells were grown in TSB broth, suspended in Butterfield's phosphate buffer (pH 7.2) to selected levels and released into the chamber through a commercial nebulizer. Saturated magnesium chloride and sodium chloride were used to adjust the chamber to 40 – 45% and 75 – 80% relative humidity, respectively. Aerosol particle sizes released from the nebulizer at both moisture conditions ranged from 0.2  $\mu\text{m}$  – 1  $\mu\text{m}$ . Aerosols and air were mixed thoroughly through an air pump.

The settling rate of aerosol-borne *L. monocytogenes* released into the chamber at  $10^8$  cfu/liter air was monitored. TSAYE plates at the bottom of the chamber were opened for 15 min each at 0, 0.5, 1, 1.5, 2, 2.5, and 3 h after releasing *L. monocytogenes*. *L. monocytogenes* cells settled on TSAYE plates were undetected 3 h after releasing.

Settling of *L. monocytogenes* onto food at different concentrations under both RH conditions was studied. Ham slices were heat-treated for 5 min at 71°C, aseptically cut to fit into Petri dishes (100 mm x 15 mm) and placed into the chamber and the ham was exposed. Bacteria were released into the chamber at 30 – 40,  $10^2$  –  $10^3$ ,  $10^5$ , and  $10^7$  cfu/liter air at both RH conditions. Plates were covered at 5, 30, 60, 120, 180, and 240 min after release of aerosolized *L. monocytogenes*. Ham slices in plates were enriched in UVM broth, transferred to Fraser broth incubated appropriately, streaked onto MOX agar, and confirmed by an enzyme-linked fluorescent antibody assay. The incidence of positive ham slices/total ham slices was recorded. Similar patterns were obtained for both RH conditions. No *L. monocytogenes* contaminated ham slices were detected at 30 – 40 cfu/liter of air at both RH conditions.

Cell injury in the air was determined by recovering *L. monocytogenes* cells on TSAYE, MOX and TSAYE/MOX overlay technique. Nearly all cells were injured as evidenced by an absence of colonies on MOX but very high numbers of cells were recovered on the TSAYE and TSAYE/MOX overlay.

Preliminary results from this research revealed that *L. monocytogenes* may not be recovered 4 h after releasing into the bioaerosol chamber. Ham was not contaminated when exposed to levels close to what may occur under industrial conditions, although direct fallout of *L. monocytogenes* onto ham was observed at higher cell numbers of *L. monocytogenes*. Air and aerosols appear to be unlikely sources of direct measurable *L. monocytogenes* of product in meat processing facilities.

### **SENSITIVITY OF *ESCHERICHIA COLI* O157:H7 TO COMMERCIAL ALKALINE CLEANERS AND SUBSEQUENT RESISTANCE TO HEAT AND SANITIZERS (M. Sharma and L. R. Beuchat)**

Exposure to bacterial cells to extreme pH may make bacterial cells more resistant to subsequent extreme pH environments that would otherwise be lethal. Acid-adapted stationary phase cells of *Escherichia coli* O157:H7 are more resistant than unadapted cells to heat. Increased heat tolerance of acid-adapted cells correlates well with the synthesis of heat-shock proteins by acid-adapted non-pathogenic *E. coli* but little is known about the survival and potential for induction of cross protection of *E. coli* O157:H7 upon exposure to alkaline environment. The pathogen may, however, be exposed to alkaline conditions in a variety of pre- and post-processing and handling environments resulting from the use of alkaline cleaners and sanitizers in food processing plants and the food service industry.

The *rpoS* gene has been reported to play an important role in the survival of *E. coli* and *Salmonella* cells exposed to chemical and physical stresses. *E. coli* O157:H7 cells deficient in the expression of the *rpoS* gene were more susceptible to acidic, osmotic, and heat stresses than were wild-type cells. The *rpoS* gene may also aid in survival of *E. coli* O157:H7 in high pH environments, providing cells with a simple mechanism to tolerate extreme alkaline conditions they may encounter in the gastrointestinal system of a host. However, studies evaluating the role of *rpoS* in *E. coli* O157:H7 upon exposure to alkaline cleaners and sanitizers commonly used in food

processing environments have not been reported. The objective of this study was to determine the survival characteristics of *E. coli* O157:H7 upon exposure to alkaline cleaners commonly used in food processing plants. Cells surviving exposure to alkaline cleaners were evaluated for changes in thermotolerance and resistance to sanitizers. The *rpoS* was examined for its role in protecting cells treated with alkaline cleaners and potential cross protection of treated cells against subsequent exposure to heat and sanitizers.

The effects of seven commercial alkaline cleaners used in the food processing industry, 0.025 M NaOH, and 0.025 M KOH on viability of wild-type (EDL 933) and *rpoS*-deficient (FRIK 816-3) strains of *E. coli* O157:H7 in logarithmic and stationary phases of growth were determined. Cells were treated at 4 or 23°C for 2, 10, or 30 min. Cleaners 2, 4, 6, and 7, which contained hypochlorite and < 11% NaOH and/or KOH (pH 11.2 – 11.7), killed significantly ( $P \leq 0.05$ ) higher numbers of cells compared to treatment with cleaner 3, containing sodium metasilicate (pH 11.4) and < 10% KOH, and cleaner 5, containing ethylene glycol monobutyl ether (pH 10.4). Treatment with KOH or NaOH (pH 11.2) was not as effective as four out of seven commercial cleaners in killing *E. coli* O157:H7, indicating that chlorine and other cleaner components have bactericidal activity at high pH. Stationary phase cells of strain EDL 933 that had been exposed to cleaner 7 at 4 or 23°C and strain FRIK 816-3 exposed to cleaner 7 at 23°C had significantly ( $P \leq 0.05$ ) higher  $D_{55^\circ\text{C}}$  values than cells of FRIK 816-3, indicating that exposure to cleaner 7 confers cross protection to heat. Cells of EDL 933 treated with cleaner 7 at 12°C showed significantly higher  $D_{55^\circ\text{C}}$  values than cells of FRIK 816-3, indicating that *rpoS* may play a role in cross protection. Stationary phase cells treated with cleaner 5 or cleaner 7 at 4 or 12°C were not cross protected against subsequent exposure to sanitizers containing quarternary ammonium compounds or sodium hypochlorite, or to cetylpyridinium chloride and benzalkonium chloride.

