

SANITATION

CONTROL OF *LISTERIA MONOCYTOGENES* BY COMPETITIVE EXCLUSION BACTERIA IN FLOOR DRAINS OF A POULTRY PROCESSING PLANT (T. Zhao, M. P. Doyle, T. C. Podtburg, P. Zhao, B. E. Schmidt, D. A. Baker, B. Cords, and R. Howell)

Recent outbreak investigations revealed that contamination of the environment of food processing facilities is a primary source of *L. monocytogenes* in many commercially prepared ready-to-eat (RTE) processed foods. Studies have revealed that certain strains of *L. monocytogenes* can become well established in a food processing facility in locations such as floor drains and remain members of the resident microbial flora for months or years. Although significant improvements in plant layout and equipment design, and procedures for cleaning and sanitizing have been made, it is believed that *L. monocytogenes* will continue to be introduced into the environment in which RTE foods are exposed for further processing and packaging.

Controlling the widely distributed psychrotrophic *L. monocytogenes* in food processing facilities has been a formidable challenge for the entire food industry, from the smallest to the largest food processor. Besides the pathogen's widespread occurrence in nature, it is nonfastidious, grows at refrigeration temperature, forms protective biofilms, and thrives in moist environments. Floor drains in food processing facilities are a particularly important niche for its existence and can be a control point of contamination for the processing plant environment and food products.

Decontaminating floor drains of *Listeria* is especially challenging because when entrapped in a biofilm, listeriae are afforded unusual protection against disinfectants and treatment available to control pathogens on environmental surfaces. Our goal was to characterize microorganisms that would thrive in combination with *Listeria* within its biofilm at a wide range of temperatures that occur in food processing facilities (especially at refrigeration conditions) and would compete to control listerial growth and possibly eliminate the pathogen.

Based on previous studies, two competitive exclusion (CE) bacteria, strains C-1-92 (*Lactococcus lactis* subsp. *lactis*) and 152 (*Enterococcus durans*) were selected for use to treat floor drains in a raw meat poultry processing facility to reduce/eliminate *Listeria monocytogenes*. In cooperation with industry partners, Ecolab and Gold Kist, Inc., a poultry processing plant located in Athens, Georgia was chosen for the field trial. Before treatment, the floor drains were tested every two weeks for five times plus one time after sanitation of the plant for *Listeria*. Samples were collected from five locations in each of five floor drains. The sampling locations included (a) bottom of drain, (b) right side of drain, (c) left side of drain, (d) under metal support of drain, and (e) surface of the floor within 1 foot of the drain.

The average number of *Listeria* in floor drains sampled at six different times (at 2-week intervals) ranged from 3.3 to 4.0 log₁₀ cfu/cm² for drain #1, from 4.2 to 5.4 log₁₀ cfu/cm² for drain #3, from 3.4 to 4.5 log₁₀ cfu/cm² for drain #4, from 3.2 to 4.2 log₁₀ cfu/cm² for drain #6, and from 6.1 to 8.2 log₁₀ cfu/cm² for drain #8. Following these samplings, 10⁷ CE bacteria/ml in foam-based medium developed by Ecolab (St. Paul, MN) were applied to the floor drains daily for four times during the first week (Monday through Thursday). Then the treatment was applied twice a week (Tuesday and Thursday) for the next three weeks. Samples were collected for *Listeria* count determinations once a week for the five weeks following application. The average number of *Listeria* (log₁₀ cfu/cm²) in samples collected one week after treatments were applied were <1.7 (positive only by selective enrichment) for drain #1, 2.0 to 3.7 for drain #3, 0 (negative by selective enrichment) to <1.7 for drain #4, 0 (all negative by selective enrichment) for drain #6 and 2.2 to 4.6 log₁₀/cm² for drain #8. Results indicate that application of these two CE bacteria can greatly reduce *Listeria* cell numbers in floor drains at 2 to 30°C in a poultry processing facility.

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN BIOFILM ON STAINLESS STEEL BY TREATMENT WITH AN ALKALINE CLEANER AND A BACTERIOPHAGE (M. Sharma, J.-H. Ryu, and L.R. Beuchat)

Biofilms formed by *Escherichia coli* O157:H7 on inadequately cleaned and sanitized contact surfaces may be a source of contamination of ground beef and deli meat in processing facilities as well as in food service settings. Refrigeration temperatures under these conditions provides opportunities for *E. coli* O157:H7 originating from fecal material on carcasses and hides to survive, attach to hydrophilic surfaces such as stainless steel, and become persistent. Cells attached to surfaces or enmeshed in biofilms may have altered sensitivities to cleaners and sanitizers compared to sensitivities of planktonic cells. *Salmonella* in biofilms has been reported to be spp. were more resistant than planktonic cells to acidic challenge, hypochlorite, and iodophors treatments. Biofilms may protect cells through a combination of mechanisms, including diffusional resistance of the EPS matrix, chemical and enzymatic inactivations of sanitizers and disinfectants, physiological changes in cells, and the induction of stress responses in cells. Strongly alkaline cleaners containing with hypochlorite have been shown to be effective in killing planktonic cells of *E. coli* O157:H7 but little is known about the ability of alkaline cleaners to inactivate biofilms of *E. coli* O157:H7 in biofilms.

Bacteriophages have been applied to various poultry products and fresh-cut produce for the purpose of inactivating foodborne pathogens. A lytic bacteriophage specific for *Salmonella* Enteritidis was shown to reduce populations of the pathogen counts on chicken skin. Reductions were increased as the multiplicity of infection (MOI, the number of phage particles needed to infect one bacterial cell) value increased. Treatment with bacteriophage has been reported to reduce populations of *Salmonella* on vegetable seed sprouts and fresh-cut honeydew melons.

We undertook a study to determine the effectiveness of an alkaline cleaner used in food processing plants and a lytic bacteriophage specific for *E. coli* O157:H7 in killing wild type and *rpoS*-deficient cells of the pathogen in a biofilm. Wild type and *rpoS*-deficient cells were attached to and developed biofilms on stainless steel coupons (ca. 7 – 8 log₁₀ cfu/coupon) at 22°C for 96 h in M9 minimal salts media (MSM) with one transfer to fresh media. Coupons were treated with 100% (pH 11.9, with 100 µg/ml free chlorine) and 25% with working concentrations of a commercial alkaline cleaner used in the food industry (pH 11.9), chlorine solutions (50 and 100 µg/ml free chlorine), or sterile deionized water (control) free chlorine solutions at 4°C for 1 and 3 min. Treatment with 100% alkaline cleaners reduced populations by 5 – 6 log₁₀ cfu/coupon for a significantly ($P \leq 0.05$) reduction compared to treatment with water more than other treatments. Initial populations (2.6 log₁₀ cfu/coupon) of attached cells of both strains attached to SSC's were reduced by 1.2 log₁₀ cfu/coupon when treated with bacteriophage KH1 (7.75 x 10⁷ log₁₀ PFU_{pfu}/ml) for up to 4 days at 4°C. Low populations (2.7 – 2.8 log₁₀ cfu/coupon) of wild type and *rpoS*-deficient biofilms cells in biofilms that had developed for 24 h at 22°C were not decreased by more than 1 log₁₀ cfu/coupon when treated with KH1 (7.5 log₁₀ 3.4 x 10⁷ PFU_{pfu}/ml) at 4°C. Results showed that higher numbers of cells of *E. coli* O157:H7 in biofilms are killed by treatment with an alkaline cleaner than with hypochlorite alone, possibly through a synergistic mechanism of alkaline pH and hypochlorite. Populations of cells attached on coupons were reduced by treating with bacteriophage but cells enmeshed in biofilms are protected. The alkaline involving pHs, in combination with hypochlorite, in a commercial cleaner are responsible for killing *E. coli* O157:H7 in biofilms. Treatment with bacteriophage KH1 may reduce populations of cells attached to coupons attached cells but not cells in biofilms.

Dry, fermented salami and sausage have been implicated as vehicles in outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) infections. Exposure of foodborne pathogens to acid or alkali stress may cross protect cells against other stresses. Highly alkaline cleaners are used to clean smokehouses, commercial ovens, and high pressure and mechanized systems. The widespread use of these cleaners in pre- and post-processing environments may result in adaptation of foodborne pathogens

to alkaline pH and cross protection to subsequent stress environments. The objective of this research was to determine the survival characteristics of *E. coli* O157:H7 cells exposed to alkaline cleaners, inoculated into sliced roast beef and hard salami, and stored at various temperatures. The *rpoS* gene was examined for its role in initiating mechanisms resulting in the protection of cells against treatment with alkaline cleaner and subsequently promoting survival and growth in roast beef and salami.

Survival and growth of wild-type (EDL 933) and *rpoS*-deficient (FRIK 816-3) strains of *E. coli* O157:H7 after exposure to an alkaline cleaner for 2 min and inoculation into roast beef (pH 6.3) and hard salami (pH 4.9) at low (0.003 – 0.52 cfu/g) and high (0.69 – 31.5 cfu/g) populations were determined. Roast beef was stored at 4 and 12°C; salami was stored at 4, 12, and 20°C. At 4°C, untreated cells of both strains showed greater reductions in populations in salami than in roast beef during a 21-day storage period. Populations of treated and untreated cells recovered from roast beef and salami stored at 4°C on tryptic soy agar were significantly ($P \leq 0.05$) higher than on sorbitol MacConkey agar, indicating that a portion of the cells was injured. Treated and untreated cells grew in roast beef at 12°C. Growth of treated cells of the FRIK 816-3 strain in roast beef at 12°C was significantly slower than that of the EDL 933 strain. Populations of both strains decreased at different rates in salami stored at different temperatures (20°C > 12°C > 4°C). *E. coli* O157:H7 strain EDL 933 grew more rapidly at 20°C in a slurry (pH 5.97) prepared from stored salami (17 days at 20°C) on which *Penicillium chrysogenum* had grown than in slurry (5.23) prepared from salami showing no mold growth. Within 2 - 3 days, populations were ca. 3 log cfu/ml higher in slurry made from infected salami compared to control salami. Results indicate that treatment of *E. coli* O157:H7 with an alkaline cleaner for 2 min does not impair resuscitation and growth of surviving cells in roast beef at 12°C. Cross protection of cells exposed to an alkaline cleaner against subsequent stress conditions imposed by roast beef and salami stored at 4°C was not evident in either of the test strains.