

ESCHERICHIA COLI O157:H7

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 in these environments provide 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 more resistant than planktonic cells to acidic challenge, hypochlorite, and iodophors. Biofilms may protect cells through a combination of mechanisms, including diffusional resistance of the EPS matrix, chemical and enzymatic inactivation of sanitizers and disinfectants, physiological changes in cells, and the induction of stress responses in cells. Strongly alkaline cleaners containing 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 *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 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 medium. Coupons were treated with 100% (pH 11.9, 100 µg/ml free chlorine) and 25% working concentrations of a commercial alkaline cleaner used in the food industry, chlorine solutions (50 and 100 µg/ml free chlorine), or sterile deionized water (control) at 4°C for 1 and 3 min. Treatment with 100% alkaline cleaner reduced populations by 5 – 6 log₁₀ cfu/coupon for a significant ($P \leq 0.05$) reduction compared to treatment with water. Initial populations (2.6 log₁₀ cfu/coupon) of attached cells of both strains were reduced by 1.2 log₁₀ cfu/coupon when treated with bacteriophage KH1 (7.7 log₁₀ 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 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 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 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 but not cells in biofilms.

SURVIVAL AND GROWTH OF *ESCHERICHIA COLI* O157:H7 IN ROAST BEEF AND SALAMI AFTER EXPOSURE TO AN ALKALINE CLEANER (M. Sharma, G. M. Richards, and L. R. Beuchat)

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.

TREATMENTS FOR CONTROL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN DRINKING WATER CONTAMINATED WITH RUMEN CONTENT OR FECES (P. Zhao, M. P. Doyle, T. Zhao, J. W. West, J. Bernard, and H. Cross)

E. coli O157:H7 has emerged in the last 10 years as an important foodborne pathogen with an estimated 73,000 cases annually in the U.S. Cattle are the major reservoir and studies revealed that when present in cattle drinking water, *E. coli* O157:H7 was disseminated to other cattle using the contaminated water source. Hence, drinking water for cattle is an important vehicle of *E. coli* O157:H7 transmission. Studies indicate that once contaminated in the drinking water of a cattle farm, *E. coli* O157:H7 can survive for many months.

A variety of treatments have been evaluated for their efficacy in killing *E. coli* O157:H7 in drinking water contaminated with rumen content or cattle feces. Results revealed that most had minimal effect on killing *E. coli* O157:H7 because these treatments were neutralized by organic materials present in the rumen content or feces. The objective of this study was to identify practical treatments to eliminate or control *E. coli* O157:H7 in drinking water simulating on-farm conditions.

Survival of *E. coli* O157:H7 in water containing rumen content at different water:rumen content, *E. coli* O157:H7 cell numbers, and temperatures was determined. At 21°C, *E. coli* O157:H7 inoculated at a

high inoculum ($10^{5.8}$ cfu/ml) survived for 8, 15, 23, >56 and 24 weeks and at a low inoculum ($10^{2.9}$ cfu/ml) survived for 8, 11, 10, 11 and 10 weeks at a water:rumen content ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively.

Different treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate and competitive inhibition *E. coli* were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm) and ozone treatment (22-24 ppm at 5°C or 8-12 ppm at 21°C) of water had minimal effect on killing *E. coli* O157:H7 in the presence of rumen content at ratios of 50:1 and higher. Treatment by competitive inhibition *E. coli* in water with rumen content also had minimal effect on *E. coli* O157:H7 counts compared with untreated controls. Four chemical treatment combinations including: (a) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid (Treatment A); b) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate (Treatment B); (c) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid (Treatment C); (d) 0.1% lactic acid, 0.9% acidic calcium sulfate and 100 ppm chlorine dioxide (Treatment D) were highly effective at 21°C in killing *E. coli* O157:H7, O26:H11 and O111:NM/ml in water heavily contaminated with rumen content (ratio of 10:1 water:rumen content, v/w) or feces (ratio of 20:1, water:feces, v/w). Among them, Treatments A, B and C killed >5 log₁₀ *E. coli* O157:H7, O26:H11 and O111:NM/ml within 30 min in water containing rumen content. For Treatment D, *E. coli* O157:H7, O26:H11, and O111:NM were reduced within 30 min by 2.8, 4.3, and 3.2 log cfu/ml in water containing rumen content, respectively, and by 3.5, 4.9, and 4.6 log cfu/ml in water with feces, respectively.

Cattle fed ad libitum water containing Treatment A, C, or control (untreated water) for two treatment periods at 7-day increments drank an average of 15.2, 13.8, and 30.3 L/day, respectively. Cattle provided water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 L/day. The amount of water consumed for all water treatments was significantly different from the control, and there were no significant differences among water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow body weight (BW) was converted to MBW ($BW^{0.75}$), and intake of water per MBW was calculated. Treatment effects for water intake/MBW were similar to those for total water intake. Because water intake was substantially reduced when treated with the chemicals described above, optimal on-farm use of such treatments would be periodic, rather than continuous. In addition, application of chemicals to drinking water systems followed by flushing to remove or dilute the chemicals after 30 minutes of exposure is recommended.

ATTACHMENT AND BIOFILM FORMATION BY *ESCHERICHIA COLI* O157:H7 ON STAINLESS STEEL AS INFLUENCED BY EXOPOLYSACCHARIDE PRODUCTION, NUTRIENT AVAILABILITY, AND TEMPERATURE (J.-H. Ryu, H. Kim, and L. R. Beuchat)

Escherichia coli O157:H7 is known to produce extracellular polysaccharides (EPS). The presence of EPS surrounding cells provides a physical barrier to protect cells against environmental stresses. Exopolysaccharides are involved in various steps of biofilm formation, including development of a conditioning film, adhesion of cells, and the formation of microcolonies and a three-dimensional biofilm structure. The EPS matrix not only increases the resistance of cells in biofilms against environmental stresses but also facilitates availability of nutrients. Exopolysaccharide clearly acts as a conditioning film in the construction of the three dimensional structure of biofilm but there is some controversy concerning the role EPS plays in initial adhesion of bacteria to surfaces. There is evidence that EPS enhances as well as inhibits the initial attachment of microorganisms on inert surfaces. A reason for this discrepancy may be that EPS can act as an adhesive or antiadhesive, depending on the attachment surface and medium in

which cells are suspended. The role EPS produced by *E. coli* O157:H7 plays in the initial attachment and biofilm formation by cells on stainless steel surfaces has not been described. The objectives of this study were to determine if EPS production by *E. coli* O157:H7 affects the initial attachment of cells on stainless steel surfaces and to determine the effects of nutrient availability and temperature in attachment and subsequent biofilm formation by wild-type and EPS-producing strains of *E. coli* O157:H7 on stainless steel coupons (SSC).

The influence of EPS production, nutrient availability, and temperature on attachment and biofilm formation by *E. coli* O157:H7 strains ATCC 43895 (wild type) and 43895-EPS (extensive EPS-producing mutant) was investigated. Cells grown on heated lettuce juice agar (HLJA) and modified tryptic soy agar (mTSA) were suspended in phosphate-buffered saline (PBS). SSC were immersed in the cell suspension (10^9 cfu/ml) at 4°C for 24 h. Biofilm formation by cells attached to SSC as affected by immersing in 10% tryptic soy broth (TSB), lettuce juice broth (LJB), and minimal salts broth (MSB) at 12 and 22°C was studied. A significantly lower number of strain 43895-EPS cells, compared to strain ATCC 43895 cells, attached to SSC during a 24-h incubation (4°C) period in PBS suspension. Neither strain formed biofilm on SSC subsequently immersed in 10% TSB or LJB but both strains formed biofilms in MSB. Populations of attached cells and planktonic cells of strain ATCC 43895 gradually decreased during incubation for 6 days in LJB at 22°C but populations of strain 43895-EPS remained constant for 6 days at 22°C, indicating that the EPS-producing mutant, compared to wild type strain, has higher tolerance to the low-nutrient environment presented by LJB. It is concluded that EPS production by *E. coli* O157:H7 inhibits attachment on SSC and reduced nutrient availability enhances biofilm formation. Biofilm formed under conditions favorable for EPS production may protect *E. coli* O157:H7 against sanitizers used to decontaminate lettuce and produce processing environments.

BIOFILM FORMATION BY *ESCHERICHIA COLI* O157:H7 ON STAINLESS STEEL AND ITS RESISTANCE TO CHLORINE AS AFFECTED BY EXOPOLYSACCHARIDE AND CURLI PRODUCTION (J.-H. Ryu and L. R. Beuchat)

A biofilm can be defined as a sessile bacterial community of cells that live attached to each other and to surfaces. Attachment and biofilm formation by foodborne pathogens and spoilage microorganisms on food contact surfaces in processing plants are a public health and cross-contamination concern. Biofilms can also form on the surfaces of containers used for harvesting, transporting, and displaying foods at the retail level and develop on food surfaces. *Escherichia coli* O157:H7 can form biofilm on stainless steel and sloughing of cells may result in cross-contamination of foods during processing. The resistance of bacterial cells embedded in biofilm against environmental stresses such as sanitizers routinely used in the food industry can be dramatically increased. *E. coli* O157:H7 has also been shown to produce curli, a thin, coiled fimbriae-like extracellular structure. Understanding the role of EPS and curli produced by *E. coli* O157:H7 on attachment, biofilm formation on foods and food contact surfaces, and protection of cells against sanitizers commonly used in processing plants and foodservice settings would provide fundamental information of practical significance when developing intervention strategies to eliminate or control the pathogen. The objectives of this study were to determine if the production of EPS and curli by *E. coli* O157:H7 affects attachment and biofilm formation on stainless steel, and to determine the influence of EPS and curli production on resistance of cells to chlorine.

The resistance of *E. coli* O157:H7 strains ATCC 43895-, 43895-EPS (an exopolysaccharide [EPS] overproducing mutant), and ATCC 43895+ (a curli-producing mutant), was studied. Planktonic cells of strains 43895-EPS and/or ATCC 43895+ grown under conditions supporting EPS and curli production, respectively, showed the highest resistance to chlorine, indicating that EPS and curli afford protection. Planktonic cells (ca. $9 \log_{10}$ cfu/ml) of all strains, however, were killed within 10 min by treatment with 50 µg/ml chlorine. Significantly lower numbers of strain 43895-EPS, compared to strain ATCC 43895-, attached to stainless steel coupons but the growth rate of strain 43895-EPS on coupons was not significantly different than that of strain ATCC 43895-, indicating that EPS production did not affect cell

growth during biofilm formation. Curli production did not affect the initial attachment of cells to coupons but did enhance biofilm production. The resistance of *E. coli* O157:H7 against chlorine increased significantly as cells formed biofilm on coupons; strain ATCC 43895+ was most resistant. Population size of strains ATCC 43895+ and ATCC 43895- in biofilm formed at 12°C were not significantly different but cells of strain ATCC 43895+ showed significantly higher resistance compared to cells of strain ATCC 43895-. These observations support the hypothesis that the production of EPS and curli increase the resistance of *E. coli* O157:H7 to chlorine.

FATE OF ACID-ADAPTED AND NONADAPTED *ESCHERICHIA COLI*, *LISTERIA MONOCYTOGENES*, AND *SALMONELLA* ON GROUND OR WHOLE BEEF JERKY (R. A. Morrow, M. A. Harrison, and J. A. Harrison)

The objective of this study was to determine the fate of acid-adapted and nonadapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on ground and whole beef jerky strips during the home-style jerky process. Each organism and meat type was compared separately and analyzed using a split-plot experimental design. To achieve acid-adapted and nonadapted cultures, each pathogen was grown in tryptic soy broth with and without dextrose, respectively. After incubation, the pH of the acid-adapted culture was 4.88 and the nonadapted was 6.97. Inoculated strips were dried in a vertical dehydrator with an air temperature of 60.0°C. For ground beef strips, samples were taken at time 0, 2, 4, 6, and 10 h. After 10 h, population reductions of acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 5.86 and 5.30, 4.73 and 3.96, and 4.28, and 4.51 log₁₀, respectively. When population reductions were compared for the same organism, there was no significant difference ($p>0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on ground beef strips. Whole beef strips were sampled after inoculation, after marination, and at 4, 8, 12, and 14 h. Population reductions after 14 h for acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 5.25 and 5.13, 4.85 and 4.82, and 4.81 and 4.87 log₁₀, respectively. When population reductions were compared for the same organism, there was no significant difference ($p>0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on whole beef strips.

PERSISTENCE OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA* NEWPORT, AND *SALMONELLA* POONA IN THE GUT OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*, AND TRANSMISSION TO PROGENY AND UNINFECTED NEMATODES (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Free-living, bacterivorous nematodes are attracted to areas in soil in which large populations of bacteria are present, so their presence on produce grown in these soils would be likely. *Caenorhabditis elegans* has been reported to feed on human pathogenic bacteria such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Poona, *Salmonella* Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*, as well as on *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Ingestion of *S. Typhimurium*, *S. aureus*, and *P. aeruginosa* shortens the life span of *C. elegans*. We hypothesized that free-living nematodes may ingest human enteric pathogens present in soil matrices and harbor them in their gut. Ingested pathogens may then remain in the gut and be protected against environmental stresses imposed by desiccation or sanitizers used to decontaminate raw fruits and vegetables, even after the worm has died. A preliminary objective of this study was to confirm that *C. elegans* ingests *E. coli* O157:H7 and salmonellae. Major objectives were to determine persistence characteristics of the pathogens in the gut after ingestion, the effects of temperature and relative humidity on survival and growth of ingested cells, and transmission of ingested *Salmonella enterica* serotype Newport to adult progeny of *C. elegans* and to uninfected worms.

Worms were fed cells of a non-pathogenic strain of *E. coli* (OP50), *E. coli* O157:H7, *S. Newport*, and *Salmonella* Poona, followed by incubating at 4, 20, or 37°C for up to 5 days. Initial populations of

ingested pathogens significantly increased by up to 2.93 log₁₀ cfu/worm within 1 day at 20°C on K agar and remained constant for an additional 4 days. When worms were placed on Bacto agar, populations of ingested pathogens remained constant at 4°C, decreased significantly at 20°C, and increased significantly at 37°C within 3 days. Worms fed *E. coli* OP50 or *S. Newport* were incubated at 4 or 20°C at relative humidities of 33, 75, or 98% to determine survival characteristics of ingested bacteria. Fewer cells of the pathogens survived incubation at 33% relative humidity compared to higher relative humidities. Populations of ingested *E. coli* OP50 and *S. Newport* decreased by up to 1.65 and 3.44 log₁₀ cfu/worm, respectively, in worms incubated at 20°C and 33% relative humidity. Placement together on K agar of adult worms, labeled with green fluorescent protein (gfp) in the pharynx area, that had ingested gfp-labeled *S. Newport* and uninfected wild type worms resulted in transfer of the pathogen to gut of wild type worms. *S. Newport* was isolated from *C. elegans* two generations removed from exposure to the pathogen. Results of these studies show that *C. elegans* may serve as a temporary reservoir of foodborne pathogens, and could perhaps be a vector for contaminating preharvest fruits and vegetables, thus potentially increasing the risk of enteric infections associated with consumption of raw produce.

