Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs)
2004 Highlights

ECOLOGY

SHEDDING OF FOODBORNE PATHOGENS BY *CAENORHABDITIS ELEGANS* IN COMPOST-AMENDED AND UNAMENDED SOIL (G. L. Anderson, S. J. Kenney, L. R. Beuchat, and P. L. Williams)

Nematodes are the most abundant soil metazoa and play an important role in soil ecology. Free-living forms that feed on bacteria respond rapidly to new nutrient sources and have a major impact on soil microfauna. Free-living nematodes harbor ingested bacteria, including human pathogens, and grow in manure-amended soils. Thus, the potential for nematodes to act as vectors of human pathogenic bacteria may be increased if manure or improperly treated compost is used as soil amendments. We conducted a study to determine the time course over which *Caenorhabditis elegans* sheds ingested *Escherichia coli* O157:H7, *Salmonella* Poona, *Listeria monocytogenes*, and a non-virulent strain (OP50) of *E. coli*. The ability of *C. elegans* to survive and reproduce on these bacteria was determined. The effect of adding turkey manure compost to soil on populations of *C. elegans* and *E. coli* O157:H7 ingested by worms before inoculation of soil was studied.

Synchronous populations of *C. elegans* were fed for 24 h on confluent lawns of nalidixic acid-adapted bacteria. *C. elegans* shed viable cells of ingested bacteria on tryptic soy agar supplemented with nalidixic acid (50 µg/ml) (TSAN) throughout a 5-h post-feeding period. *C. elegans* persisted for up to 10 days by feeding on bacteria that had been shed and grew on TSAN. Eggs harvested from *C. elegans* cultured on shed foodborne pathogens had the same level of viability as those collected from *C. elegans* grown on shed *E. coli* OP50. After 6 - 7 days, 78, 64, 64, and 76% of eggs laid by *C. elegans* that had fed on *E. coli* O157:H7, *S.* Poona, *L. monocytogenes*, and *E. coli* OP50, respectively, were viable. Worms fed *E. coli* O157:H7 were inoculated into soil and soil amended with turkey manure compost. Populations of *C. elegans* persisted in compost-amended soil for at least 7 days but declined in unamended soil. *E. coli* O157:H7 was detected at 4 and 6 days post inoculation in compost-amended and unamended soil, and in unamended soil inoculated with *E. coli* OP50. Populations of *E. coli* O157:H7 in soil amended with turkey manure compost were significantly ($\alpha = 0.05$) higher than those in unamended soil. Results indicate that *C. elegans* can act as a vector to disperse foodborne pathogens in soil, potentially resulting in increased risk of contaminating the surface of pre-harvest fruits and vegetables.

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 $\log_{10}$ 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 $\log_{10}$ 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.

MIGRATION OF *CAENORHABDITIS ELEGANS* TO MANURE AND MANURE COMPOST AND POTENTIAL VECTORING OF *SALMONELLA* NEWPORT TO FRUITS AND VEGETABLES (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

It is not uncommon for animal manure and manure compost to be applied to cropland soil as fertilizers. The application of manure and manure compost to soil may attract nematodes that feed on bacteria. Free-living, microbivorous nematode populations have been reported to increase in soils to which cattle manure slurry has been applied. The extent to which various types of manure and manure composts are incorporated into the soil can influence populations of nematodes. Sand homogeneously amended with a humus-litter mixture has been reported to support higher populations of *Caenorhabditis elegans* compared to sand containing isolated patches of the humus-litter mixture. It is hypothesized that free-living nematodes such as *C. elegans* and possibly other genera may ingest human pathogens occasionally found in the soil and transport them through the soil matrix. As a worm migrates through soil it may come in contact with external tissues of plants, either by attraction mechanisms or by random chance. A study was undertaken to determine if *C. elegans* is attracted to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella* Newport. Survival and reproduction of *C. elegans* in the same matrices not inoculated with *S.* Newport were investigated. Movement of *C. elegans* to lettuce, strawberries, and carrots on an agar medium and the ability of the nematode to transport *S.* Newport in soil to the surface of produce were also studied.

C. elegans moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of *C. elegans* in test materials was not affected by the presence of *S.* Newport. Bovine manure and bovine manure compost inoculated with *S. enterica* serotype Newport (8.6 $\log_{10}$ cfu/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with *C. elegans*. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20°C for up to 10 days. In the system using soil inoculated with *C. elegans, S.* Newport initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when *C. elegans* was not present in the soil.

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Results indicate that *C. elegans* has the potential for transporting pathogens in soil to the surface of preharvest fruits and vegetables in contact with soil.

INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIOIDES* AND *PENICILLIUM EXPANSUM*, AND ASSOCIATED MIGRATION OF *SALMONELLA* POONA INTO EDIBLE TISSUES (G. M. Richards and L. R. Beuchat)

Cantaloupe fruits are often in contact with the ground during their development, enhancing the potential for contamination by microorganisms capable of causing human diseases. They are susceptible to postharvest fungal rots, especially under warm, wet conditions. Complete loss of the commodity occurs when one or a few fungal pathogens invade and begin to breakdown the tissues. We undertook a study to determine if the growth of two molds known to cause decay of cantaloupes, *Cladosporium cladosporioides* and *Penicillium expansum,* in wounds on rinds facilitate migration of *Salmonella* Poona into sub-surface mesocarp tissues.

Two phytopathogens, *Cladosporium cladosporioides* and *Penicillium expansum,* in wounds on cantaloupe rinds, were studied to assess their potential to facilitate migration of *S.* Poona into sub-surface mesocarp tissues. Wounded sites in cantaloupe rind were inoculated with *S.* Poona only, *S.* Poona and mold simultaneously, or mold followed by *S.* Poona 3 days later. A cylindrical plug (ca. 3 cm diameter and 4 cm deep) of inoculated tissue extending from the rind surface into edible tissues was removed and cut transversely into four segments (0 –1, 1 – 2, 2 – 3, and 3 – 4 cm) representing distances from the rind surface. Regardless of the type of inoculum or the time of storage subsequent to inoculation, the pH of the tissues was significantly higher ($P \leq 0.05$) as the distance from the rind surface increased. Test microorganisms and naturally-occurring microorganisms on the rind surface which were introduced into internal tissues during wounding, as well as physiological changes in cantaloupe tissue, contributed to these changes. *C. cladosporioides* and *P. expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20°C for 10 days. *S.* Poona persisted and grew in wounds on rinds on inoculated cantaloupe incubated at 20°C. Recovery of *S.* Poona from tissues 3 – 4 cm below the inoculated wound supports the hypothesis that it can migrate from the site of inoculation into adjacent mesocarp tissues. Survival and migration of *S.* Poona into the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and, to a lesser extent, *P. expansum*. Consumption of cantaloupes from which diseased tissue has been removed is not advisable because *S.* Poona and perhaps other enteric pathogens may still be present in remaining tissues.

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METABIOTIC ASSOCIATIONS OF MOLDS AND *SALMONELLA* POONA ON INTACT AND WOUNDED CANTALOUPE RIND (G. M. Richards and L. R. Beuchat)

Several national and international outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes. Cantaloupe fruits may be in direct contact with the ground during their development on long, running, non-climbing vines that are prostrate on the soil. The growth habit of cantaloupes enhances the potential for fruits to be contaminated by pathogens that may be present in the soil. Postharvest handling may also bring cantaloupes in direct contact with various sources of foodborne pathogens. Mesocarp tissues of fruits are particularly subject to contamination when rind surface integrity is compromised by disease, bruising, cutting, or peeling. Infection of cantaloupes by plant pathogenic fungi and contamination with foodborne pathogenic bacteria may occur before harvesting, at the time harvest, during handling, storage, transport, and marketing, or after purchase by the consumer. The behavior of foodborne pathogens such as *Salmonella* on or in cantaloupes as affected by metabiotic activities of plant pathogens has not been investigated. The objective of this study was to examine the association between selected molds pathogenic to cantaloupes and *Salmonella* Poona on the surface of intact rind and in wounds in the rind. Changes in pH caused by growth of molds were monitored, as were survival and growth of *S.* Poona in co-infected tissue as affected by temperature.

We tested proteolytic activity and measured changes in the pH of cantaloupe rind caused by growth of *Alternaria alternata, Cladosporium cladosporioides, Epicoccum nigrum, Geotrichum candidum*, and *Penicillium expansum.* Survival and growth characteristics of *S.* Poona co-infected with each mold on the surface rind and in wounded rind tissue as affected by temperature were determined. *C. cladosporioides, G. candidum*, and *P. expansum,* but not *A. alternata* and *E. nigrum*, showed proteolytic activity on agar media containing gelatin and/or casein, with concurrent increases in pH, thus favoring survival and growth of salmonellae. Intact and mechanically wounded tissues of cantaloupe rinds were inoculated with a five-strain mixture of *S.* Poona and/or test mold. Five inoculation schemes were used: mold only, *S.* Poona only, mold and *S.* Poona simultaneously, mold then *S.* Poona 3 days later, and *S.* Poona then mold 3 days later. The pH of cantaloupe rinds inoculated with molds and stored at 20°C for 14 days was significantly higher ($P \leq 0.05$) than on day 0. Only the pH of rinds inoculated with *C. cladosporioides* or *G. candidum* was significantly higher ($P \leq 0.05$) on day 21 than on day 0, when cantaloupes were stored at 4°C. An initial population of *S.* Poona increased from 3.3 $\log_{10}$ cfu/sample (ca. 7 cm^2) of cantaloupe rind to populations as high as 9.5 $\log_{10}$ cfu/sample during storage at 20°C for up 14 days, regardless of co-inoculation with molds. Populations of *S.* Poona decreased or remained constant at 4°C for up to 21 days. Results demonstrate that persistence and growth of *S.* Poona on intact, wounded, and decaying cantaloupe rind is not affected by the presence of molds.

MICROFLORA ON GEORGIA-GROWN CANTALOUPES RELATED TO PACKAGING AND HANDLING PRACTICES (E. D. Akins, M. A. Harrison, and W. C. Hurst)

In recent years, there has been foodborne illness outbreaks associated with the consumption of cantaloupe. Contamination of cantaloupes with microorganisms could occur anywhere from the field to the packing line. Cantaloupes are handled and packed differently in various regions of the United States. Typically, California cantaloupes are field packaged while in Georgia they are brought to sheds, washed, and packed. The objective of this study was to determine the number of microbes that are on cantaloupes coming out of the field, after washing, and after packing.

Four Georgia growers with packing facilities, who use slightly different variations in product handling, were visited four times during the 2004 cantaloupe harvest season. For each visit, 20 cantaloupes were sampled from each of the following steps: after transport from the field, after washing, and after packing. The washing method varied among the facilities with 2 using chlorinated water, 1 using heated water, and 1 using a combination of heat and chlorinated water.

There was a slight, but significant decrease in microbial populations between the samples from the field and after washing in dump tanks at the two farms using chlorinated treatments. Exposing cantaloupes to water between 41-50°C (105-122°F) water for 5-10 minutes did not result in a significant change in microbial populations. Similarly, microbial populations on the cantaloupes after packing were approximately the same as that on the prewashed cantaloupes. Washing, chlorination and hot water treatments applied under actual field packing conditions in Georgia do not significantly affect the total aerobic populations on cantaloupes.

***LISTERIA MONOCYTOGENES* SURVIVAL IN REFRIGERATOR DILL PICKLES** (J. Kim, E. M. D'Sa, M. A. Harrison, J. A. Harrison, and E. L. Andress)

Listeria monocytogenes can survive and grow in refrigerated foods with pH levels of approx. 4.0-5.0 and salt concentrations of 3-4%. Refrigerator dill pickles fit this description. Contamination of this product with *L. monocytogenes* could cause serious problems since these items are not heated prior to consumption. This study determined whether *L. monocytogenes* survives and grows in refrigerator dill pickles at three salt levels (1.3, 3.8, and 7.6%). Cucumbers were inoculated with *L. monocytogenes*. Brine mixtures were poured over the cucumbers and they were held at room temperature for one week and then stored under refrigeration for up to 3 months. The pH and percent NaCl and total aerobic,

psychrotrophic, lactic acid bacteria, and *Listeria* counts were measured following the addition of brine, at 2, 4, and 7 days, during storage at room temperature, and then later at weekly intervals during refrigerated storage. There was a rapid decrease in pickle pH after four days at room temperature (from 6.2-6.3 to 4.4-4.8) followed by a gradual decrease. The percent NaCl in the pickles increased only slightly while held at room temperature from 0 to 0.101, 0.234, and 0.448% in 1.3, 3.8, and 7.6% salt mixtures, respectively. The initial *Listeria* population was 6-7 log cfu/in^2 on the surface and 4-5 $\log_{10}$ cfu/g internally. There was approximately 1 log increase during fermentation at room temperature followed by a population decline during refrigerated storage, with a greater decrease in the pickles with the highest NaCl content. Populations of total aerobes and lactic acid bacteria increased. Based on old recommendations consumption of refrigerator dill pickles could typically be anytime after 3 days of refrigerated storage. Since *L. monocytogenes* may still be viable well after this point, there is a food safety risk and no recommendations to prepare this product in the home should be distributed.

THE MICROBIAL COMMUNITY STRUCTURE OF THE CHICKEN INTESTINE (M.D. Lee)

The intestinal microbiota is composed of a complex bacterial community that is influenced by the host's age, physiology, and diet. Consequently, the microbial community structure is important in the maintenance of intestinal health. The long-term goal of our research is to identify the mechanisms involved in the interaction between the bacterial intestinal communities and intestinal health. Colonization with certain bacterial species is necessary for normal development of the mucosa and mucosal immune responses. Compositional analysis of bacterial communities is increasing in interest because of new technologies that enable broad surveys of diverse environments. There are 2 methods used to assay the composition of microbial communities. The culture method is recognized to have significant weaknesses due to the inability to culture many of the abundant organisms in some environmental samples. A molecular method, analysis of 16S rRNA genes present among the community DNA, is currently used because of this gene's discriminatory ability in identifying bacteria to the genus, and frequently species, level.

Using this molecular approach, we have characterized the bacterial community of the intestine of chickens fed a corn-soy diet over a standard commercial grow out period. In this study, we sequenced 1200 clones obtained from the community DNA of the ileum and cecum of broilers at different ages. The cecum contained a very high diversity of lactobacilli and clostridia as well as streptococci, enterococci, proteobacteria, and *Bacterioides*. We detected many species of bacteria in each cecal community DNA library and the abundance of each ranged from 1-56% of the total clone population. The ileum contained some of the same phylotypes of bacteria as the cecum, however, the diversity and abundance was much different with the ileal community being dominated by lactobacilli. The composition of the community also varied considerably by age of the bird. In addition, the *Clostridiaceae* were significantly abundant in the ileum during all sampling times, however, the birds did not exhibit any gross signs of intestinal inflammation.

A molecular approach to characterizing the microbial community of the intestine is revealing weaknesses in our view of the bacteria composing this community. We have commonly viewed the clostridia as pathogens and have failed to evaluate their potential role as intestinal symbionts. A reevaluation of the composition of the commensal community of the chicken will aid in new paradigms of evaluating intestinal health and probiotic formulation.

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 60.0ºC. 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 $\log_{10}$, 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 $\log_{10}$, 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.

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 12ºC 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.

The role of aerosol in transmission of microorganisms (including *Listeria*) to ready-to-eat meat/poultry products. (L. Ma, C. M. Lin, Z. Yan, J. Kornacki, O. Oyarzabal, and M.P. Doyle)

Airborne contamination of *Listeria monocytogenes* in food processing facilities may or may not be an important contributing factor in disseminating *L. monocytogenes* in such facilities. However, aerosol studies in food processing plants have been limited by lack of a suitable surrogate microorganism for *L. monocytogenes*. The objectives of this study were to investigate the potential of using *Jonesia denitrificans* as a surrogate for aerosol studies of *L. monocytogenes* and to study the role of aerosols in the transmission of microorganisms (including *L. monocytogenes*) to ready-to-eat meat/poultry products.

The settling rates of aerosol-borne *J. denitrificans* released into a bioaerosol chamber were determined. Studies revealed that settling rates depend on particle size and relative humidity of the environment. Larger particles settled from the air more rapidly than smaller particles, with 5-µm particles

completely settled out of the air within a few minutes of releasing and 0.3-µm particles remaining airborne ($<1 \log_{10}$ reduction) for 4 hours. In most instances, relative humidity (RH) at 40 or 75% had minimal effect on settling rates, although settling rates of *J. denitrificans* were slightly greater at 75% RH than at 40% RH. Overall, *J. denitrificans* had similar settling rates as *L. monocytogenes* (previous studies).

The contamination level of *J. denitrificans* on turkey meat following its aerosolization in the bioaerosol chamber was similar to that of *L. monocytogenes* which was dependent on initial inoculum cell numbers, exposure time, and relative humidity. The greater the number of cells in the aerosol, the greater the number of contaminated turkey samples and the less exposure time for contamination to occur. No turkey samples were *J. denitrificans* or *L. monocytogenes* positive within 4 hours exposure time when the initial cell number was $\leq 2.5 \times 10^2$ or $\leq 1.5 \times 10^2$ cfu/L air, respectively, and all samples were positive within 5 to 30 minutes of exposure when cell inoculum populations were $\geq 3.5 \times 10^5$ cfu/L air. More samples were positive in the 75% RH environment when the inoculum was 10^3 cfu/L air but relative humidity had little influence on the number of contaminated samples for higher or lower levels of inoculum. Both the detectable cell numbers of *J. denitrificans* and *L. monocytogenes* on positive samples of non-cured turkey meat were generally low, ranging from 1 to 12 cfu per three slices. These results suggest that even when relatively large cell numbers are in the aerosols in a room, relatively small numbers contaminate the surface of products during a short exposure.

Releasing *J. denitrificans* at 10^3 cfu/L as an aerosol into a deboning room of a poultry processing pilot facility revealed that the distance from the air conditioning units from which the bacteria were aerosolized influenced the level of *J. denitrificans* contamination that occurred. The greatest degree of contamination occurred at 100 to 150 cm from the air conditioners, and least at 50 and 250 cm from the units. For samples obtained at 100 cm, the greatest average number of *J. denitrificans* on agar media was 2.4×10^2 cfu/plate, and greatest percentage of meat samples positive at a sampling distance was 40%. Results indicate that releasing as an aerosol at a high population (10^3 cfu/L), *J. denitrificans* can contaminate agar plate and meat surfaces at a range of 250 cm from air conditioning units with the greatest degree of contamination occurring within 100 to 150 cm of an air conditioning unit. Interestingly, swab sampling of environmental surfaces of the deboning room immediately after aerosolizing *J. denitrificans* yielded negative results; indicating *J. denitrificans* is not a good environmental survivor.

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