

SALMONELLA SPP.

COMPETITIVE EXCLUSION BACTERIA AGAINST *SALMONELLA* COLONIZATION IN CHICKENS (G. Zhang, L. Ma, and M. P. Doyle)

Poultry and poultry products are important sources of human foodborne salmonellosis. Newly hatched chickens are highly susceptible to infection and colonization by *Salmonella*. Pretreatment of chicks with microflora obtained from the GI tract of adult healthy chickens can protect chicks from *Salmonella* infection; however, undefined cultures from chicken GI tract may transfer bacterial pathogens or bacteria that confer antibiotic resistance into new flocks. The objective of this project is to develop a defined competitive exclusion product that can reduce or eliminate *Salmonella* colonization in poultry.

The first step was to isolate from healthy chickens competitive exclusion bacterial candidates having antimicrobial activity to *Salmonella* in vitro. Using selective and non-selective media, 143 isolates with inhibitory activity to six *Salmonella* strains of poultry origin (2 *Salmonella* Enteritidis, 1 *Salmonella* Typhimurium, 1 *Salmonella* Heidelberg, 1 *Salmonella* Kentucky, and 1 *Salmonella* Senftenberg) were obtained from 9 donor chickens.

Seventeen trials have been carried out to test the effectiveness of many of the competitive exclusion bacterial candidates in reducing *Salmonella* colonization of chicks. The general design of these trials was similar for all, although there were slight variations between different trials. Each trial included 18 treatments, with 10 to 25 chicks per treatment. Undiluted overnight CE bacteria (approximately 10^7 - 10^8 cfu/chick) were fed to chicks at day-of-hatch and the following day. Chicks were challenged with different strains of *Salmonella* (*S.* Typhimurium, *S.* Kentucky, and *S.* Enteritidis) (ca. 10^4 cfu/chick) when they were 3 days old. They were necropsied at ca. 10 days of age. Cecal contents from each chick were enumerated on BGA plates; at the same time, they were enriched in RV broth and streaked onto BGA plates. Results were obtained for (a) the percentage of *Salmonella*-positive birds out of the total number tested in each treatment and (b) the level of *Salmonella* carriage (expressed as the geometric mean of the counts per gram of cecal content for all chicks tested in the group). CE cultures grown in MRS broth before feeding to chicks yielded inconsistent results. Modifying MRS broth to provide nutritional conditions more similar to that of the intestinal tract of chicks were *Salmonella*-positive. The effectiveness of many of the CE bacteria against *Salmonella* in chickens varied; however, six CE isolates showed consistent effectiveness against *Salmonella* colonization in chickens in repeated trials. They reduced *Salmonella* carriage in cecal content by more than 2 log₁₀ cfu/g on average for three trials. The percentage of chickens *Salmonella*-positive after treatment with these CE bacteria was ca. 30%, whereas ca. 80% of control (no CE bacteria treatment) were *Salmonella*-positive. Currently, we are testing different combinations of these six CE bacteria to identify the best combinations. Cultural conditions for growing the best performing CE bacteria will be optimized, and these CE bacteria will be identified and further characterized for phenotypic and genotypic properties.

FATE OF *SALMONELLA* IN NUTRIENT BROTH AND ON THE SURFACE OF RAW OYSTERS AS AFFECTED BY CHITOSAN

(W. Klypradit, P. Chhabra, and Y. W. Huang)

This study was initiated to determine the effect of chitosan, a modified carbohydrate polymer derived from chitin, on growth inhibition of *Salmonella* spp. as tested in nutrient broth and in shelled oysters. Three levels of chitosan, 1, 2, and 3%, were prepared in 0.5% acetic acid and the pH adjusted to 6.5 by adding NaOH solution. Pure cultures of *Salmonella* were inoculated into nutrient broth containing the chitosan in acetic acid or applied to oysters coated with the chitosan/acetic acid material. All nutrient broth samples were kept at 7°C for a period of 48 h while oysters were kept at 4°C for 15 d. Stored inoculated broths and oysters were sampled at 12-h and 5-d intervals, respectively. Initial counts of all broth samples averaged 6.0×10^8 cfu/ml. A 7-log₁₀ reduction occurred in the presence of chitosan/acetic acid after 48 h incubation at 7°C while samples containing 0.5% acetic acid alone exhibited a 2-3 log₁₀ reduction. The initial counts in oysters averaged 1.8×10^5 cfu/g. After a 5-d storage period,

Salmonella populations started to decline. After a 15-d storage period, *Salmonella* populations declined 1, 3, and 2- \log_{10} in oysters exposed to 1, 2, and 3% chitosan, respectively. No reduction in *Salmonella* population was observed in oysters coated with an acetic acid solution. Overall, 2% chitosan was considered the best treatment level for inactivation of *Salmonella* on raw oysters.

RADIO-FREQUENCY HEATING OF ALFALFA SEED FOR REDUCING HUMAN PATHOGENS
(S. O. Nelson, C.-Y. Lu, L. R. Beuchat, and M. A. Harrison)

The production of sprouts from alfalfa and other seeds for human consumption is a substantial industry; however, there have been several outbreaks of illness associated with sprouts, and contamination by *Salmonella* and *Escherichia coli* O157:H7 has been identified as the cause. Contaminated seed used for sprouting is considered the most likely source of these human pathogens. No sprout-related illness attributable to *Listeria monocytogenes* has yet been documented, but this pathogen also poses a potential threat. Because most of the outbreaks of infections have been attributed to contaminated sprouting seed, several methods have been studied for decontaminating seed. Treatment of alfalfa seed in hot water at 54°C significantly reduced seed viability. Several aqueous solutions of chemicals, including chlorine, chlorine dioxide, hydrogen peroxide, trisodium phosphate, ethanol, peracetic acid, and some commercial fruit and vegetable produce wash solutions have been studied for decontaminating alfalfa seed. None of these treatments eliminated *E. coli* O157:H7 or *Salmonella* from alfalfa seed intended for sprouting.

Earlier research has shown that radio-frequency (RF) and microwave dielectric heating treatments are effective for increasing the germination percentage of alfalfa seed lots containing high percentages of hard seed. Hard seeds occur naturally and are viable seeds with seed coats that are impermeable to water. Therefore, they will not germinate promptly when planted, but they may germinate several weeks, months, or years later when the seed coat becomes permeable through natural processes. Mechanical scarification of such seed lots to increase germination is common practice for alfalfa, but the abrasive process scratches the seed coat thus providing a favorable environment for bacterial attachment, which may make sanitization with liquids more difficult. Thus, it appeared reasonable to explore the possible use of dielectric heating for reduction of bacterial populations on alfalfa seed, especially since the improvement of germination and subsequent sprout yield can be achieved without mechanical abrasion of the seed coat. Similar consistent increases in alfalfa seed germination through hard seed reduction have been achieved by dielectric heating at frequencies of 5, 10, 39, or 2,450 MHz. Treatment at 39 MHz was selected for this study because of equipment availability and because it provides a more uniform electric field for exposure of the samples than is commonly available in microwave ovens.

The potential for controlling human bacterial pathogens on alfalfa seed used in the production of sprouts by dielectric heating was studied by experimental exposure of alfalfa seed artificially contaminated with *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* to RF dielectric heating treatments at 39 MHz and different electric field

**FATE OF *ESCHERICHIA COLI* O157:H7, *Listeria monocytogenes*,
AND *SALMONELLA* SPP. IN REDUCED SODIUM HOME-STYLE BEEF JERKY**
(M. A. Harrison)

Interest in low-sodium food products necessitates re-examination of home preservation processes relying in part on salt for antimicrobial effects. The fates of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. in reduced sodium home-style beef jerky was determined using different salt levels in ground or whole beef strips. Samples were either dried in a 60°C dehydrator or heated to an internal temperature of 71.1°C prior to drying in a 60°C dehydrator. Populations were determined at time 0 and 2 h intervals until dry. Population reductions were greater in ground beef with non-reduced salt levels compared to that with reduced salt levels, and in most cases, greater reduction (1.0 – 1.5 \log_{10}) was observed for ground beef strips heated prior to drying. For dried whole jerky strips, there generally were no significant differences ($p > 0.05$) in pathogen populations between the non-reduced and reduced salt marinade in the end product. The results from this study support the importance of the antimicrobial effect of sodium chloride in particular products on the pathogens used in this experiment.

**ATTRACTION OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*,
TO FOODBORNE PATHOGENIC BACTERIA, AND ITS POTENTIAL AS A VECTOR OF *SALMONELLA* POONA
FOR PREHARVEST CONTAMINATION OF CANTALOUPE**
(K. N. Caldwell, G. L. Anderson, P. L. Williams, and L. R. Beuchat)

Soil is a source of microbial contamination of fruits and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. Free-living, microbivorous nematodes are among the primary grazers of bacteria in soils and also have potential to serve as vectors of microorganisms, including enteric pathogens, to the surface of fruits and vegetables. Most nematologists do not attach particular importance to free-living nematodes as vectors of plant pathogens. However, a critical examination of the role nematodes may play in plant and perhaps human diseases has been suggested. *Caenorhabditis elegans*, a microbivorous, free-living nematode, has been used extensively in biological studies. Feeding primarily on bacteria, the adult worm lives approximately 2 weeks under optimal environmental conditions. The worm is routinely cultured in the laboratory on *Escherichia coli* OP50, a uracil-deficient non-pathogenic strain that grows slowly on K agar but serves as a nutrient source for multiplication and reproduction. The objectives of this study were to determine the propensity of *C. elegans* to migrate toward three human enteric pathogens and cantaloupe juice, as well as its survival and reproductive behavior in the presence of these pathogens. The potential of *C. elegans* as a vector to transport *Salmonella* in soil to the surface of cantaloupe rind was also investigated.

The propensity of *C. elegans* to be attracted to seven strains of *Escherichia coli* O157:H7, eight serotypes of *Salmonella*, six strains of *Listeria monocytogenes*, and cantaloupe juice was investigated. Adult worms (20 - 30) were placed on the surface of K agar midway between a 24-h bacterial colony and 10 µl of uninoculated tryptic soy broth (TSB) or cantaloupe juice positioned 1.5 cm apart. The number of nematodes that migrated to the colony, TSB, or cantaloupe juice within 5, 10, 15, and 20 min at 21°C was determined, followed by incubating plates at 37°C for up to 7 days to determine the ability of *C. elegans* to survive and reproduce in bacterial colonies. The nematode was attracted to colonies of all test pathogens, and survived and reproduced within colonies for up to 7 days. *C. elegans* was not attracted to cantaloupe juice. The potential of *C. elegans* to serve as a vector to transport *Salmonella* Poona to cantaloupe rind was investigated. Adult worms that had been immersed in a suspension of *S. Poona* were deposited 1 or 3 cm below the surface of soil on which a piece of cantaloupe rind was placed. The rind was analyzed for the presence of *S. Poona* after 1, 3, 7, and 10 days at 21°C. The presence of *S. Poona* was evident more quickly on rind positioned on soil beneath which *C. elegans* inoculated with *S. Poona* was initially deposited compared to rind on soil beneath which *S. Poona* alone was deposited. The time required to detect *S. Poona* on rind was longer when the rind was placed 3 cm above the inoculum, compared to 1 cm. Free-living nematodes may play a role in the preharvest dispersal of incidental human pathogens in soil to the surface of raw fruits and vegetables in contact with soil during development and maturation, as evidenced by the behavior of *C. elegans* as a test model.

**INTESTINAL CARRIAGE OF *CAMPYLOBACTER* AND *SALMONELLA* IN TURKEYS
IN RESPONSE TO SUB-THERAPEUTIC LEVELS OF ANTIMICROBIALS IN FEED
(N. A. Cox, S. E. Craven, M. T. Musgrove, M. E. Berrang, and N. J. Stern)**

Since the 1950s, antimicrobials have been added to poultry feed at sub-therapeutic levels to minimize illness and promote growth. Despite the benefits to the agricultural industry and domestic animals, there are fierce debates worldwide on whether or not this practice carries a consequence in terms of human health. Turkeys and broilers provided these additives have increased weight gain, muscle yield, and feed conversion, in part due to decreases in diseases such as coccidiosis and necrotic enteritis. Benefits achieved by adding these compounds to animal feeds are attributed in part to a shift in the gut microflora. However, studies have been published in which it was determined that competitive exclusion cultures, administered to birds to control colonization by human pathogens such as *Salmonella*, can be negatively affected by antimicrobials commonly used in poultry rations. Other published studies have reported an increase in *Salmonella* levels when experimentally challenged birds were fed diets containing low levels of antimicrobials. This study demonstrated that although naturally occurring populations of *Campylobacter* were virtually unaffected by antimicrobial feed additives, *Salmonella* populations were significantly decreased when commercial turkeys were fed rations containing flavomycin, virginiamycin, or monensin.

**INTERACTION OF A FREE-LIVING SOIL NEMATODE, *CANENORHABDITIS ELEGANS*,
WITH SURROGATES OF FOODBORNE PATHOGENIC BACTERIA
(G. L. Anderson, K. N. Caldwell, L. R. Beuchat, and P. L. Williams)**

The agricultural impacts of plant and animal parasitic nematodes have long been recognized and, by virtue of their effects on fruit and vegetable production, have been extensively studied. Comparatively little is known regarding the impact of free-living microbiovorous nematodes on produce production and safety, although they are the most abundant and wide spread soil mesofauna. The association of free-living nematodes and various genera of bacteria has been studied. While it is recognized that free-living nematodes avoid certain bacteria, it is clear that they do not uniformly avoid foodborne pathogens. Two human enteric pathogens, *Salmonella* and *Shigella*, are reportedly ingested and defecated by free-living saprozoic nematodes and *Salmonella* Typhimurium is known to infect the free-living soil nematode *Caenorhabditis elegans*. From these reports, it appears that free-living nematodes may be important as vectors of pathogenic bacteria, including some forms capable of causing human disease.

Soil is a source of microbial contamination of fruit and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. In a survey of vegetables for the presence of amoebae and *Salmonella*, nematode eggs and larvae have been recovered using a naccoal-ether method. The recovery of nematodes from uncooked vegetables indicates that agronomic conditions and marketing practices may be conducive to the survival of nematodes on fresh produce. This also indicates that if free-living nematodes are present on raw produce, they may serve as vehicles for contamination with pathogenic bacteria, either by contact with their surface or via eggs or voided material from their gastrointestinal tract.

We undertook a study to evaluate the interaction of *C. elegans* with bacterial surrogates for foodborne pathogens occasionally occurring or persisting in soil. Nematode/bacterial interactions were characterized to determine the propensity of young adult worms to be attracted to bacterial colonies, to compare the feeding and development of young adult worms cultured on this diverse group of bacteria, and to examine the dispersal of bacteria by *C. elegans* following feeding on monoxenic cultures. We evaluated the association between a free-living soil nematode, *C. elegans*, with *Escherichia coli*, an avirulent strain of *S. Typhimurium*, *Listeria welshimeri*, and *Bacillus cereus*. On an agar medium, young adult worms quickly moved toward colonies of all four bacteria; over 90% of 3-day adults entered colonies within 16 min after inoculation. After 48 h, worms moved in and out of colonies of *L. welshimeri* and *B. cereus*, but remained associated with *E. coli* and *S. Typhimurium* colonies for at least 96 h. Young adult worms fed on cells of the four bacteria suspended in K medium. Worms survived and reproduced using nutrients derived from all test bacteria, as evidenced by eggs laid by second generation worms after culturing for 96 h. Development was slightly slower in worms fed on Gram-positive bacteria compared to Gram-negative bacteria. Worms fed for 24 h on bacterial lawns formed on tryptic soy agar dispersed bacteria over a 3-h period when transferred to a bacteria-free agar surface. Results suggest that *C. elegans* and, perhaps, other free-living nematodes are potential vectors for both Gram-positive and Gram-negative bacteria, including foodborne pathogens in soil.

SURFACE MATERIAL, TEMPERATURE AND SOIL EFFECTS ON THE SURVIVAL OF SELECTED FOODBORNE PATHOGENS IN THE PRESENCE OF CONDENSATE

(J. Allan, J. L. Kornacki, and Z. Yan)

Survival of foodborne pathogens in food processing facilities is affected by factors including surface materials, nutrients, moisture and temperatures. The effects of surface-type [stainless steel, Delrin[®] (DuPont) acetal resin, and fiberglass reinforced plastic wall paneling (FRP), and mortar surfaces], soil, and temperature on the survival of *Listeria monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* were evaluated in the presence of condensation. Surface coupons soiled and non-soiled with sterile porcine serum were exposed to cell suspensions made from five-strain cocktails of each organism (10^7 cfu/ml) in Butterfield's phosphate buffer (BPB) and incubated for 2 h at 25°C allowing attachment of cells. Three surfaces, stainless steel, Delrin and FRP, were included in the same experiment. The coupons were rinsed to remove unattached cells, incubated at either 4°C or 10°C under condensate-forming conditions, and sampled at six time intervals over a 15-day period. For enumeration, cells were removed from the coupons by vigorous shaking in 100 ml BPB with 3 g of glass beads and the BPB was plated on tryptic soy agar with 0.6 % yeast extract. The results showed that stainless steel did not support the survival of *Listeria* as well as acetal resin or FRP. Acetal resin and stainless steel were less supportive of *Salmonella* than FRP. All three surfaces supported the survival of *Yersinia* over the 15-day trial equally at 10°C. Temperature had little effect on survival of *Listeria* or *Salmonella* across all three surfaces. However, *Yersinia* displayed growth on FRP at 10°C, but death at 4°C. Serum had a protective effect on *L. monocytogenes* on all surfaces, but did not affect survival of *Salmonella* or *Yersinia* on stainless steel, acetal resin, or FRP.

Since mortar surface is very different from the three surfaces described above, it was tested separately. The method to enumerate bacterial cells on the mortar surface involved applying sonication to remove bacterial cells and determining the cfu/coupon at 9 to 10 sampling periods over a total of 120 h. In general, the mortar surface had a significant inhibitory effect against all the bacteria tested compared to the three surfaces described above because of alkaline pH (increased to pH 11 within 6 h) when submerged in BBP. *Listeria* and *Salmonella* survived better on mortar than *Yersinia* throughout the 120-h incubation period, partially due to the alkaline resistance of *L. monocytogenes* and *Salmonella* spp. Serum had a protective effect on the survival of all three organisms. Differences in temperature did not affect the survival of *Salmonella* or *Yersinia*, whereas populations of *L. monocytogenes* declined more rapidly at 10°C than at 4°C after 24 h.

**EFFECT OF INOCULUM SIZE, RELATIVE HUMIDITY, STORAGE TEMPERATURE, AND RIPENING STAGE
ON THE ATTACHMENT OF *SALMONELLA* MONTEVIDEO TO TOMATOES AND TOMATILLOS
(M. H. Iturriaga, E. F. Escartin, L. R. Beuchat, and R. Martinez-Peniche)**

Bacterial survival and growth on and in fruits and vegetables depends, in part, on their ability to attach to surfaces. Sessile microorganisms have advantages in that they are more difficult to mechanically remove from surfaces and are more resistant to disinfectants compared with planktonic cells. It has been observed that attachment of bacterial cells is affected by several factors, including the medium in which they are grown, motility, temperature, length of contact time, and production of extracellular polysaccharides. Information describing the attachment of human pathogens on fruits and vegetables is limited; however, the process appears to be similar to that of adhesion of plant pathogenic and non-pathogenic bacteria on leaves. One possible intervention to minimize the risk of human infections associated with the consumption of raw fruit and vegetables contaminated with foodborne pathogens is the application of chemical treatments. The increased resistance of attached microorganisms to sanitizing agents may partially explain the observed limited effect of disinfection treatments. A clearer understanding of the process of bacterial adhesion to fruit surfaces is essential to devising more effective methods for removal. The objective of this research was to evaluate the effects of inoculum size, relative humidity, ripening stage, and storage temperature on the attachment of *S. Montevideo* to the surface of tomatoes and tomatillos.

The influence of inoculum populations and environmental factors on attachment of *Salmonella* Montevideo to the surface of tomatoes and tomatillos was evaluated. To study the effect of inoculum size, red, ripe tomatoes were spot inoculated with bacterial suspensions (10^5 and 10^8 cfu/fruit) and stored at 22°C under 100% relative humidity. The effects of temperature (12, 22, and 30°C) and relative humidity (75, 85, and 97%) on attachment of the pathogen (10^7 cfu/fruit) on tomatoes (red and green) and ripe tomatillos were also evaluated. Inoculated fruit were stored for 90 min at all combinations of temperature and relative humidity, and after washing with water the number of cells attached to the surface was determined. *S. Montevideo* attached to the surface of tomatoes within 90 min. A direct correlation between the number of attached cells and the population in the inoculum was observed. The percentage of cells that attached immediately after inoculum was approximately 0.3% for the test products. After storage for 90 min at various temperature/relative humidity conditions, the number of adhering cells ranged from 4.0 to 5.4 \log_{10} cfu/fruit (1.2% of inoculum). Both the type of product and the temperature/relative humidity condition had a significant ($P \leq 0.05$) effect on attachment of *S. Montevideo* to the surfaces of tomatoes and tomatillos. Scanning electron micrographs of the cuticles of inoculated washed tomatoes and tomatillos revealed typical skin cell patterns, and only a few randomly dispersed *S. Montevideo* were observed. Deposition *S. Montevideo* on the surface of tomatoes and tomatillos may result in attachment and subsequent colonization under suitable conditions.

**FACTORS AFFECTING SURVIVAL, GROWTH, AND RETRIEVAL OF *SALMONELLA* POONA
ON INTACT AND WOUNDED CANTALOUPE RIND AND IN STEM SCAR TISSUE
(L. R. Beuchat and A. J. Scouten)**

The first documented outbreak of salmonellosis in the U.S. that was associated with consuming cantaloupes was reported in 1990. Consumption of cantaloupes imported from Mexico and Central America was linked to 295 cases of *Salmonella enterica* serotype Chester infections in 30 states. In the summer of 1991, more than 400 cases of *Salmonella enterica* serotype Poona infections in the U.S. and Canada were associated with the consumption of cantaloupe. Twenty-five cases of *Salmonella enterica* serotype Saphra infections in the U.S. were linked to

cantaloupes imported from Mexico in 1997 and 22 cases of *Salmonella enterica* serotype Oranienburg infections in Canada in 1998 were attributed to consuming cantaloupes. Subsequent outbreaks of *S. Poona* infections in the U.S. and Canada have been associated with eating cantaloupes grown in Mexico. A survey to determine the presence of foodborne pathogens on fresh produce imported to the U.S. revealed that 11 of 151 (7.3%) cantaloupes were contaminated with *Salmonella* or *Shigella*. A survey of domestically grown fruits and vegetables reported the presence of *Salmonella* on 3 of 92 (3.3%) cantaloupes. Clearly, the pathogen can be found on the surface of cantaloupes at various points postharvest and is somehow transferred to the edible portion before consumption, resulting in human infections. As few as 150 *Salmonella/cm*² of netted rind surface of cantaloupes have been shown to contaminate the edible portion upon cutting.

Several researchers have evaluated sanitizers for their efficacy in killing or removing foodborne pathogens and spoilage microorganisms on the surface of cantaloupe rind and the interior flesh. It is difficult to compare the effectiveness of various sanitizers across, and sometimes within, laboratories because of variations in methodology. Methods used to analyze cantaloupes not intentionally inoculated with *Salmonella* also vary among research and testing laboratories. While the development of a standard method to analyze raw fruits and vegetables for *Salmonella* and other pathogens has been promoted, still there are many factors that could influence the efficiency of retrieval of *Salmonella* from cantaloupes that need to be investigated before an optimum method can be recommended. We conducted a study to determine the survival and recovery of *Salmonella enterica* serotype Poona from cantaloupe rind as affected by environmental conditions between the time of contamination and analysis. Detection and enumeration of the pathogen as influenced by analytical methods were also investigated. Combinations of preenrichment broth (lactose broth or universal preenrichment broth), enrichment broth (Rappaport-Vassiliadis broth or tetrathionate broth), and selective agar medium (bismuth sulfite agar or xyline lysine desoxycholate agar) for detecting *S. Poona* on inoculated cantaloupes stored at 4°C for 7 days or 21°C for 3 days were equivalent in performance. The use of nalidixic acid as a marker in *S. Poona* and in media used to enhance detection or enumeration of the pathogen by inhibiting background microflora in sanitizer efficacy studies, for example, would not adversely affect its survival on or recovery from cantaloupes. Overall, the composition of the carrier (water or 5% horse serum, a high organic matrix) used to prepare inocula did not influence the number of *S. Poona* recovered from the intact rind surface, wounds in the surface, or the stem scar tissue. Regardless of inoculation site or composition of the carrier, populations on spot inoculated melons stored at 4°C remained constant between 2 and 24 h after inoculation. The pathogen grew within 24 h in wounds of spot- and dip-inoculated cantaloupes stored at 21 and 37°C. The addition of up to 1.0% Tween 80 to 0.1% peptone used to remove *S. Poona* from the rind surface did not adversely affect viability and may have enhanced detachment. Consideration of these observations is recommended when developing a method to test the efficacy of sanitizers in killing salmonellae on the rind surface of inoculated cantaloupes and to detect or enumerate salmonellae that may be natural contaminants.

**EVALUATION OF INOCULATION METHOD AND INOCULUM DRYING TIME FOR THEIR EFFECTS ON SURVIVAL AND EFFICIENCY OF RECOVERY OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*, AND *LISTERIA MONOCYTOGENES* INOCULATED ON THE SURFACE OF TOMATOES
(M. M. Lang, L. J. Harris, and L. R. Beuchat)**

Contamination of raw produce with pathogenic microorganisms can occur at any of several points from the field through the time of consumption. Given sufficient time and appropriate environmental conditions, pathogens can grow to populations exceeding 10⁷ cfu/g of tomato. Work has been done to define conditions that result in contamination of produce and subsequent growth of pathogens during storage. Researchers have also evaluated the effectiveness of a wide range of chemical sanitizers and physical treatments to decontaminate fresh produce. Results of studies done in different laboratories are difficult to compare, however, because of numerous variations in methodologies employed and incompleteness in describing results. A study was undertaken with the objective to evaluate procedures for inoculating *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* onto the surface of tomatoes with the goal of selecting an inoculation procedure to be used in a standard method. Dip, spot, and spray inoculation were evaluated. A second objective was to examine the effect of time between application of inoculum and analysis of tomatoes on the viability and retrievability of pathogens. Inocula applied to tomatoes were subjected to two drying times followed by either no treatment or treatment with water (control) or chlorine (200 µg/ml), then analyzed for the presence of surviving cells.

Five-strain mixtures of *Escherichia coli* O157:H7, *Salmonella*, or *Listeria monocytogenes* were applied to tomatoes by dip, spot, or spray inoculation methods. Inocula were dried for 1 or 24 h at 22°C before tomatoes were treated with water (control) or chlorine (200 µg/ml). Significantly ($\alpha = 0.05$) higher populations (cfu/tomato) of *E. coli* O157:H7 and *Salmonella* were recovered from dip-inoculated tomatoes compared to spot- or spray-inoculated tomatoes. This is attributed to larger numbers of cells adhering to tomatoes subjected to dip inoculation. Populations of *E. coli* O157:H7 and *Salmonella* recovered from spot- and spray-inoculated tomatoes containing the same initial number of cells were not significantly different. Significantly different populations of *L. monocytogenes* were recovered from inoculated tomatoes (dip > spot > spray). Populations of pathogens recovered from tomatoes were significantly higher when inocula were dried for 1 h compared to 24 h. Significant differences (water > chlorine) were observed in populations of all pathogens recovered from tomatoes treated with chlorine, regardless of inoculation method or drying time. Results indicate that inoculation method, drying time, and treatment affect survival and/or recovery of foodborne pathogens inoculated onto the surface of tomatoes. It is recommended that spot inoculation with a drying time of 24 h at 22°C be used in a standard method to determine the efficacy of chlorine and other sanitizers in killing foodborne pathogens on tomatoes.

**SURVIVAL AND RECOVERY OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*, AND *LISTERIA MONOCYTOGENES* ON LETTUCE AND PARSLEY AS AFFECTED BY METHOD OF INOCULATION, TIME BETWEEN INOCULATION AND ANALYSIS, AND TREATMENT WITH CHLORINATED WATER
(M. M. Lang, L. J. Harris, and L. R. Beuchat)**

Given sufficient time and appropriate environmental conditions after contamination, pathogens can grow to populations exceeding 10^7 cfu/g of lettuce and 10^6 cfu/g of parsley. Conditions that result in contamination of produce with pathogens and subsequent growth during storage have been described. A wide range of chemical sanitizers and physical treatments for decontamination of fresh produce has been evaluated. Results are difficult to compare, however, because of the numerous variations in methodologies. The lack of uniformity of methods used to treat produce with sanitizers and enumerate microorganisms surviving treatments makes it difficult to assess their effectiveness and establish industry recommendations and guidelines for their use. The development of a standard method would minimize or eliminate variations in methodologies used in various laboratories, thereby enabling a comparison of pathogen reductions resulting from treatment with various sanitizers. A single method may not be applicable for all fruits and vegetables but a basic test method that could be modified as necessary to accommodate natural variations in fresh and fresh-cut produce would be the goal.

One of the objectives of the study reported here was to evaluate three methods (dip, spot, and spray) for inoculating *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* onto the surface of two types of leafy salad vegetables, lettuce and parsley, with the goal of establishing a procedure for use in a standard method to test the efficacy of sanitizers. A second objective was to examine the effect of time between application of inoculum and analysis of lettuce and parsley on the viability and recovery of pathogens. Inocula applied to lettuce and parsley were subjected to two drying times, followed by either no treatment or treatment with water or chlorine then analyzed for the number of surviving cells. Inocula were dried for 2 h at 22°C or for 2 h at 22°C followed by 22 h at 4°C before treating with water (control) or chlorine (200 µg/ml). Significantly ($\alpha = 0.05$) higher populations (cfu/lettuce or parsley sample) of *E. coli* O157:H7 and *Salmonella* were recovered from dip-inoculated produce compared to spot- or spray-inoculated produce. This is attributed to larger numbers of cells adhering to lettuce and parsley subjected to dip inoculation. Populations of *E. coli* O157:H7 and *Salmonella* recovered from lettuce inoculated by spot and spray methods were not significantly different but populations recovered from spot-inoculated parsley were significantly higher than those recovered from spray-inoculated parsley, even though the number of cells applied was the same. Significantly different populations of *L. monocytogenes* were recovered from inoculated lettuce (dip > spray > spot); populations recovered from dip-inoculated parsley were significantly higher than those recovered from spot- or spray-inoculated parsley, which were not significantly different from each other. Populations of pathogens recovered from lettuce and parsley after drying inoculum for 2 h at 22°C were significantly higher than or equal to populations recovered after drying for 2 h at 22°C followed by 22 h at 4°C. Significant differences (water > chlorine) were observed in populations of all pathogens recovered from treated lettuce and parsley, regardless of inoculation method and drying time. It is recommended that spot inoculation with a drying time of 2 h at 22°C followed by 22 h at 4°C be used to determine the efficacy of chlorine and other sanitizers in killing foodborne pathogens on lettuce and parsley.

APPLICATION OF NESTED PCR TO DETECTION OF *SALMONELLA* IN POULTRY ENVIRONMENT

(J. J. Maurer)

Isolation of *Salmonella* from poultry environmental and processing plant samples requires sampling large numbers of areas within the poultry house or plant. Subsequently, this results in a large volume of work for a microbiology laboratory especially when the protocol requires inclusion of a delayed secondary enrichment for isolation of *Salmonella*. This study examined the use of PCR to identify those secondary enrichments containing *Salmonella*. The unique *Salmonella* virulence gene *invA* was chosen as the target for development of a nested-PCR because of its uniform distribution among *Salmonella* serotypes. The use of nested-PCR primers increased sensitivity of detection 100-fold, resulting in detection of as few as 4 cells. There was a statistically significant, strong positive correlation between PCR and culture results as determined by chi-squared test ($p < 0.001$) and kappa test (0.915; excellent agreement). Using PCR as a screen of primary enrichments for presumptive *Salmonella* contamination, we improved our efficiency at isolating *Salmonella* upon secondary enrichment by 20 % and no false negatives were observed. This method will not only validate the use of secondary enrichment procedures, but also reduce costs and manpower for surveillance of *Salmonella*.

DETECTION OF *SALMONELLA* AND *CAMPYLOBACTER* IN POULTRY BY PCR-ELISA

(J. J. Maurer)

Contamination of retail poultry by *Campylobacter spp.* and *Salmonella enterica* is a significant source of human diarrheal disease. Isolation and identification of these microorganisms requires a series of biochemical and serological tests. In this study, *Campylobacter ceuE* and *Salmonella invA* genes were used to design probes in PCR-ELISA, as an alternative to conventional bacteriological methodology, for the rapid detection of *Campylobacter jejuni*, *Campylobacter coli*, and *Salmonella enterica* from poultry samples. With PCR-ELISA (40 cycles), the detection limit for *Salmonella* and *Campylobacter* was 2×10^2 cfu/ml and 4×10^1 cfu/ml, respectively. ELISA increased the sensitivity of the conventional PCR method by 100- to 1,000-fold. DNA was extracted from carcass rinses and tetrathionate enrichments and used in PCR-ELISA for the detection of *Campylobacter* and *S. enterica*, respectively. With PCR-ELISA, *Salmonella* was detected in 20 of 120 (17%) chicken carcass rinses examined, without the inclusion of an enrichment step. Significant correlation was observed between PCR-ELISA and cultural methods (kappa = 0.83; chi-squared test: $p < 0.001$) with only one false negative (1.67%) and four false positives (6.67%) when PCR-ELISA was used to screen sixty tetrathionate enrichment cultures for *Salmonella*. With PCR-ELISA, we observed positive correlation between the ELISA absorbance (Optical Density_{405nm}) and *Campylobacter* cell number in carcass rinse, as determined using standard culture methods. Overall, PCR-ELISA is a rapid and cost-effective approach for the detection and enumeration of *Salmonella* and *Campylobacter* on poultry.

A RESTRICTION FRAGMENT LENGTH POLYMORPHISM BASED POLYMERASE CHAIN REACTION AS AN ALTERNATIVE TO SEROTYPING FOR IDENTIFYING *SALMONELLA* SEROTYPES

(J. J. Maurer)

The phase 1 (*fliC*) and phase 2 (*fljB*) *Salmonella* flagella genes were analyzed by RFLP-PCR to aid in the identification of different *Salmonella* serotypes. Twenty-four phase 1 flagellin and eight phase 2 flagellin genes could be differentiated among each other using restriction endonucleases *Sau3A* and *HhaI* in RFLP-PCR analysis. These flagellin genes comprise the major antigenic formulas for fifty-two serotypes of *Salmonella spp.*, which include the common serotypes found in poultry and other important food animal species. With the knowledge of the O antigen composition determined from conventional O serotyping, ninety percent of the *Salmonella* serotypes could be identified using this double restriction enzyme RFLP analysis of *fliC* and *fljB* genes. This RFLP-PCR flagellar typing scheme was successfully applied to the identification of serotype for 112 *Salmonella* isolates obtained from poultry environments. There was a significant correlation between RFLP PCR and conventional serotyping (chi-square $p < 0.001$). Overall, PCR-RFLP proved to be a fast, accurate and economic alternative approach to serotyping *Salmonella spp.* such that it is now part of diagnostic services offered to the poultry industry for identifying *Salmonella* serotypes.

**RAPID DETECTION OF POULTRY *SALMONELLA ENTERICA* SEROTYPES ENTERITIDIS, HADAR, HEIDELBERG, AND TYPHIMURIUM BY MULTIPLEX PCR TO COMMON O AND H ANTIGEN GENES AND ALLELES
(J. J. Maurer)**

There are over 2,000 different *Salmonella* serotypes but few are commonly associated with human disease. Currently, serotyping relies on a time consuming process that requires implementing a battery of antisera for typing O and H antigens of *Salmonella enterica*. A rapid multiplex PCR-based molecular serotyping scheme was developed with an ability to identify *S. enterica* serotypes Enteritidis, Hadar, Heidelberg, and Typhimurium isolated from the poultry environment. The nucleotide sequence of the O-antigen biosynthesis *rfb* operons for *Salmonella* O serogroups D1, B, C1, C2, and E1 were analyzed to identify sequences suitable for designing multiplex primer sets. By analyzing the highly variable nucleotide sequences in the central region of the phase-1 and phase-2 *Salmonella* flagella genes *fliC* and *fljB*, multiplex PCR's were designed to identify the *fliC* genes associated with the phase 1 antigen types i, g,m r, z₁₀ and the *fljB* genes belonging to two major phase 2 antigen complexes, 1,2, 1,5, 1,6, 1,7 and e,n,x, e,n,z₁₅. These antigens constitute the antigenic formulas for common clinical and poultry *S. enterica* serotypes. The multiplex PCR conditions were optimized and discrimination was achieved, identifying O or H antigen gene(s) and alleles based upon the unique sizes of the amplicons from multiplex PCR. The serotyping, multiplex PCR was comparable to the standard serological typing methods for correctly identifying *S. enterica* serotype ($\kappa = 1.00$; chi-squared test: $p < 0.001$). Coupled with primary enrichment using tetrathionate brilliant green broth, multiplex PCR correctly identified poultry samples contaminated with *S. enterica* Enteritidis, Kentucky, and Typhimurium. These multiplex PCR assays for detecting specific O and H antigen gene alleles can become a rapid and cost-effective alternative approach to serotyping for the identification of common poultry *Salmonella* serotypes. In fact, this multiplex PCR has now become part of diagnostic services offered to poultry industry for identifying *Salmonella* serotypes.

