

***ESCHERICHIA COLI* O157:H7**

SURFACE-STERILIZATION METHODS FOR *ESCHERICHIA COLI* O157:H7 ON LETTUCE (*LACTUCA SATIVA* L.)

(G. Zhang, L. Ma, L.R. Beuchat, M.C. Erickson, V.H. Phelan, and M.P. Doyle)

Many outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections have been associated with consumption of fresh-cut leafy greens in the past decade. Questions remain regarding the ability of these pathogens to become internalized within lettuce and spinach. To differentiate internalized populations from surface contamination, an effective surface-sterilization method for lettuce is needed and was the focus of this study.

Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local grocery store and cut into 3 x 3 cm pieces. Lettuce roots were purchased from a local farmer. Leaf pieces and roots were inoculated by immersing in 10⁸ CFU of a five-strain mixture of GFP-labeled *E. coli* O157:H7/ml for 10 min at room temperature. Inoculated samples were put in a laminar flow biosafety cabinet for 30 min before further treatment. Thirteen surface-sterilization methods (including sodium hypochlorite, ethanol, HgCl₂, and hydrogen peroxide) were compared for their efficacy in killing/removing *E. coli* O157:H7 on lettuce leaf and root surfaces. Treated samples were washed 5 times with sterile water, and then assayed for *E. coli* O157:H7.

Among the 13 surface-sterilization methods evaluated, *E. coli* O157:H7 was not detected by enumeration with a direct plating procedure on treated samples for 3 treatments, including 20 min with 10,000 ppm sodium hypochlorite and 2 treatments containing ethanol and HgCl₂. There were 2.8 to 4.4 CFU *E. coli* O157:H7/leaf piece or root after surface-sterilization for the other methods. Plant tissue prints on agar and enrichment culture results were consistent with enumeration results. Our overall data revealed that the best surface-sterilization method for lettuce leaves and roots was dipping in 80% ethanol for 10 s, followed by immersion in 0.1% HgCl₂ for 10 min.

SILVER NANOROD ARRAY AS A SERS SUBSTRATE FOR FOODBORNE PATHOGENIC BACTERIA DETECTION

(H.-Y. Chu, Y.W. Huang, and Y.-P. Zhao)

Surface enhanced Raman scattering (SERS) using novel silver nanorod array substrates has been used for the detection of pathogenic bacteria. The substrate consists of a base layer of 500 nm silver film on a glass slide and a layer of silver nanorod array with length of ~1 μm produced by oblique angle deposition method at a vapor incident angle of 86°. Spectra from whole cell bacteria, Generic *Escherichia coli*, *Escherichia coli* O157:H7, *E. coli* DH 5α, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhimurium*, and bacteria mixtures, have been obtained. This SERS active substrate can detect spectral differences between Gram types, different species, their mixture, and strains. Principle component analysis method has been applied to classify the spectra. Viable and nonviable cells have also been examined and significantly reduced SERS responses at major Raman bands were observed for nonviable cells. The SERS spectra of bacteria on single cell level excited at low incident laser power (12 μW) and short collection time (10 s) has also been demonstrated. These results indicate that the SERS-active silver nanorod arrays substrate is a potential analytical sensor for rapid identification of microorganisms with a minimum sample preparation procedure.

ENUMERATION OF *E. COLI* O157:H7 IN SPINACH OUTBREAK SAMPLES

(L. Ma, G. Zhang, P. Garner-Schmidt, and M. P. Doyle)

During August and September of 2006, more than 200 persons from 26 states were infected by a single strain (isolates indistinguishable by pulsed-field gel electrophoresis [PFGE]) of *E. coli* O157:H7. Fresh spinach was identified as the vehicle of the outbreak and *E. coli* O157:H7 with a PFGE pattern matching the outbreak strain was isolated from many open packages of fresh spinach consumed by patients. The objective of this study was to enumerate *E. coli* O157:H7 in outbreak-associated spinach samples obtained from patient refrigerators. A 3-tube Most Probable Number (MPN) method in combination with selective plating, multiplex PCR (targeting genes: *gad*, *eae*, *bfp*, *stx1*, and *stx2*) screening, confirmation by *E. coli* O157 latex agglutination assay, and PFGE pattern matching was used for the isolation and enumeration of the outbreak strain in the spinach samples. The estimated cell numbers in positive samples ranged from 0.61 MPN/g to 21 MPN/g. Multiplex PCR analysis revealed absence of the *stx 1* amplicon in the outbreak isolate.

**ISOLATION AND ENUMERATION OF *E. COLI* O157:H7
FROM POSSIBLE CONTAMINATED GROUND BEEF SAMPLES**
(L. Ma, G. Zhang, P. Gerner-Smidt, and M. P. Doyle)

Direct plating on selective media (TC-CHROMagar O157) and a 3-tube Most Probable Number (MPN) method were used for the isolation and enumeration of *E. coli* O157:H7 from ground beef associated with an outbreak. Presumptive-positive isolates of *E. coli* O157:H7 were confirmed molecularly (multiplex PCR targeting five genes: *gad*, *eae*, *bfp*, *stx1*, and *stx2*) and immunologically (*E. coli* O157 latex agglutination assay). Subtyping by MLVA and PFGE confirmed the isolates were indistinguishable to the outbreak strain. Three out of eight of the original samples were positive for *E. coli* O157:H7, with cell numbers of the pathogen in the positive samples being generally low, ranging from <0.3 MPN/g to 24 MPN/g by the MPN method, or from <50 CFU/g to ca. 50 CFU/g by direct enumeration. Also, multiplex PCR revealed that all of the *E. coli* O157:H7 isolates contained *eae*, *stx1* and *stx2* genes. One sample contained a large number of colonies that had the same morphology (mauve) as *E. coli* O157 on TC-CHROMagar O157 and reacted positively by the *E. coli* O157 latex agglutination assay; however, these isolates were later identified as *Serratia liquefaciens* by the API 20E test.

**ATTACHMENT AND RECOVERY OF *ESCHERICHIA COLI* O157:H7 AND A NON-PATHOGENIC SURROGATE
FROM ROMAINE LETTUCE AFTER CONTACT WITH CONTAMINATED ICE**
(J. Kim and M. Harrison)

Ice, possibly contaminated with *E. coli* O157:H7, can be used to chill romaine lettuce and maintain relative humidity during transportation. Contamination of lettuce is of concern since it is usually consumed raw or minimally processed. The potential for *E. coli* O157:H7 contamination of romaine lettuce with either ice contaminated with the pathogen or by transfer from lettuce surfaces via melting ice was determined. In order to evaluate pathogen transfer by these means in actual commercial facilities, the use of non-pathogenic surrogates is needed. A non-pathogenic *E. coli* strain was selected and compared with *E. coli* O157:H7 to determine differences and similarities in attachment to and recovery from romaine lettuce in contact with contaminated ice. *E. coli* O157:H7 distributes onto other produce layers in shipping containers due to melted ice made of contaminated water and transfers from contaminated to uncontaminated surfaces. Based on cryotolerance and cell surface characteristics, *E. coli* ATCC 25922 is a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recovery from chilled produce.

**CONTAMINATION AND POTENTIAL INTERNALIZATION OF *ESCHERICHIA COLI* O157:H7
IN PRE-HARVEST ICEBERG LETTUCE (*LACTUCA SATIVA* L.)**
(G. Zhang, L. Ma, L.R. Beuchât, M.C. Erickson, V.H. Phelan, and M.P. Doyle)

The ability of foodborne pathogens to internalize within lettuce, especially under growing conditions, is an important unanswered question in need of elucidation for risk analysis and intervention purposes. The objectives of this study were (1) to determine the effect of inoculation sites (abaxial vs adaxial leaf surfaces) on survival and internalization of *E. coli* O157:H7 in lettuce; and (2) to evaluate the vulnerability of lettuce at different ages to *E. coli* O157:H7.

Iceberg lettuce (*Lactuca sativa* L.) was grown in sandy soil in an envirotron at 23°C during the day and 7°C at night. A 5-strain mixture of GFP-labeled *E. coli* O157:H7 at 10⁶ CFU/ml in water and cow manure extract was used as inoculum. Plants were inoculated on abaxial and adaxial sides of leaf surfaces at 3, 30, and 60 days after transplantation and sampled 2 to 3 times for each inoculation treatment. At each sampling time, *E. coli* O157:H7 in soil and in/on shoots and roots were analyzed. For surface-sterilization, leaves and roots were dipped in 80% ethanol for 10 s, followed by immersion in 0.1% HgCl₂ for 10 min.

Twenty-five days after inoculation, 2 of 12 samples were *E. coli* O157:H7-positive on inoculated leaves. No *E. coli* O157:H7 was detected on inoculated leaves at 54 days. All surface-sterilized root and leaf samples were negative for *E. coli* O157:H7 regardless of plant age at inoculation, sampling time, or abaxial- or adaxial-side inoculation. Substantially more lettuce leaves inoculated on the abaxial side were *E. coli* O157:H7-positive after 3 to 25 days than those leaves inoculated on the adaxial side.

Internalization of the *E. coli* O157:H7 in iceberg lettuce by leaf inoculation did not occur. Age of lettuce plants did not affect internalization of *E. coli* O157:H7 in lettuce. Inoculated *E. coli* O157:H7 survived longer on the abaxial side of the leaves than on the adaxial side.

CONTAMINATION AND POTENTIAL INTERNALIZATION OF *ESCHERICHIA COLI* O157:H7 IN LETTUCE (*LACTUCA SATIVA* L.) BY SOIL INOCULATION

(G. Zhang, L. Ma, L.R. Beuchat, M.C. Erickson, V.H. Phelan, and M.P. Doyle)

Understanding whether internalization of foodborne pathogens occurs through plant roots will be helpful in conducting risk assessments and developing effective interventions to reduce pathogen contamination in produce. The objectives of this work were (1) to determine if internalization of *E. coli* O157:H7 through lettuce roots occurs; and (2) to determine if differences exist among *E. coli* O157:H7 isolates and lettuce types regarding *E. coli* O157:H7 internalization, survival and growth in and on lettuce plants.

Iceberg, Romaine and leaf lettuces were grown in sandy soil in an envirotron using two temperature regimes. Soil was inoculated with 5 GFP-labeled *E. coli* O157:H7 isolates individually at 10^6 or 10^3 CFU/g of soil when lettuce seedlings were transplanted. Lettuce plants were sampled 2 to 3 times after transplantation and assayed for *E. coli* O157:H7 in soil and in/on shoots and roots. For surface-sterilization, leaves and roots were dipped in 80% ethanol for 10 s, followed by immersion in 0.1% HgCl₂ for 10 min.

Results revealed that surface-sterilized leaf and root samples were negative (except for 2 root samples) for *E. coli* O157:H7. Seventeen days after transplantation and inoculation, most leaf surfaces were positive for *E. coli* O157:H7 which was likely due to cross-contamination from the inoculated soil. The 26-, 45- and 60-day samplings revealed no *E. coli* O157:H7 on leaf surfaces. Some soil and rhizosphere samples were positive for *E. coli* O157:H7 at 60 days when the trials were terminated.

In conclusion, internalization of *E. coli* O157:H7 in lettuce did not occur through the roots; however, the pathogen could survive in soil for at least 60 days. There were no differences among *E. coli* O157:H7 isolates or lettuce types with regard to *E. coli* O157:H7 internalization in lettuce.

PRE-HARVEST FACTORS AFFECTING INTERNALIZATION OF ZOONOTIC PATHOGENS INTO LETTUCE

(M.C. Erickson, J. Liao, A. Payton, C. Webb, L. Ma, G. Zhang, M. Doyle, and L.R. Beuchat)

In the past two decades, the fresh fruit and vegetable industry has rapidly evolved and contributed to increased retail and food-service sales. Accompanying this growth has been an increasing number of outbreaks associated with fresh produce consumption that has often been traced back to the farm. Potential pre-harvest vehicles for contamination of vegetables include soil amendments (manure or improperly-composted manure) or contaminated irrigation or runoff water. Based on laboratory studies, however, both surface and internalized contamination occurred when seeds or seedlings were exposed to contaminated soil or water solutions. Whether internalization occurred in older plants and the fate of any internalized populations was one of the objectives of this study.

Differences in the robustness of plant defense mechanisms that target bacterial extracellular components for subsequent subcellular compartmentalization and degradation have been suggested as one factor affecting internalized pathogen populations. Since plant stress associated with drought conditions could affect plant defensive activities, the level of internalization of zoonotic pathogens could, in turn, also be affected. Another factor that is likely to affect internalization of zoonotic pathogens is the level of indigenous microorganisms in the soil environment. Since the abundance of an indigenous population is dependent on the relative availability of nutrients, internalization of zoonotic pathogens by plants could, in turn, be affected by the level of fertility in the soil. A second objective of this study therefore addressed both the influence of plant stress and soil fertility levels on internalization of zoonotic pathogens by lettuce plants.

Green leaf lettuce (variety Two star) was grown in pots using either 0:5, 1:5 or 2:5 manure compost:top soil mixtures. Pots were held in an envirotron at 20°C during the day and 7°C at night. An inoculum mixture of green-fluorescent protein (gfp)-labeled *Escherichia coli* O157:H7 isolates or an inoculum mixture of gfp-labeled *Salmonella* spp. was prepared and added to water to give concentrations of 10^3 or 10^6 CFU/ml. Contaminated water was applied to the soil of 3- or 33-day post-transplanted plants (30-50 ml/plant) and a portion of those plants were sampled 3 days later and at 60-days post-transplantation. For a sub-group of plants exposed at 33-days post-transplantation, a reduced watering rate was applied for 2-3 weeks prior to the contamination event. With all plants, a physical barrier separated leaves and soil to prevent direct transfer of pathogens from soil to leaves. Leaves were analyzed separately from washed roots and both surface and internalized populations were enumerated for these samples. Using an ethanol and mercury chloride wash, surface sterilization of samples preceded enumeration of internalized populations.

Pre-harvest internalization of *Escherichia coli* O157:H7 or *Salmonella* spp. into roots or leaves of green leafy lettuce cultivated in a growth chamber did not occur when plants were watered with a contaminated water source. Pathogen internalization was not affected by the level of soil fertility. A 2-week period of reduced watering prior to the contamination event also did not induce internalization of pathogens. The absence of internalized populations is of merit as post-harvest interventions need only target surface contamination.

COMPETITIVE INHIBITION MICROORGANISMS FOR THE CONTROL OF ZOO NOTIC PATHOGENS IN COMPOST
(L. Ma, G. Zhang, V. Mantripragada, M. C. Erickson, and M. P. Doyle)

Indigenous microflora may play a significant role in suppression of zoonotic pathogens during static composting. The objective of this project was to isolate competitive inhibition (CI) microorganisms from static compost piles for the control of zoonotic pathogens. Compost samples from the surface of static compost piles were collected during the study of the fate of zoonotic pathogens (*E. coli* O157:H7, *Listeria innocua*, and *Salmonella* Typhimurium) in static composting of chicken litter and peanut hulls. Only samples that exhibited a large decline in inoculated pathogen populations in two consecutive sampling times were used for the isolation of CI microorganisms. Two methods were used to screen for potential CI bacteria against target pathogens (*E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*): a deferred antagonism test and a co-culture test. A total of 20 potential CI isolates against either one or all of three target pathogens were selected from 16 compost samples. Cross inhibitory activity among these isolates revealed that nine of the isolates were compatible. Characterization of these isolates by DNA sequencing of the 16S rRNA gene is currently in progress. Future studies will incorporate these isolates into cured compost materials and evaluate their potential to inhibit the growth of *Salmonella*.

