

SALMONELLA

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 5 α , *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.

ISOLATION AND ENUMERATION OF SALMONELLA FROM POT PIES FROM OREGON

(G. Zhang, L. Ma, V. Phelan, P. Gerner-Smidt, and M.P. Doyle)

During 2007, at least 272 isolates of *Salmonella* I 4,5,12:i:- with an indistinguishable pulsed field gel electrophoresis (PFGE) subtype were obtained from ill persons in 35 states. At least 65 people were hospitalized and pot pies were determined to be the vehicle of the outbreak.

Three pot pies (2 chicken pies, 1 turkey pie) were received from the Oregon Department of Public Health and assayed for *Salmonella*. Universal preenrichment broth for preenrichment and Rappaport-Vassiliadis broth (RV) and Tetrathionate broth (Hajna) (TT) for enrichment was employed for detection and enumeration of *Salmonella*. All samples were also analyzed with the BAX[®] Detection System (DuPont Qualicon).

The two chicken pot pies were negative for *Salmonella* whereas the turkey pie was contaminated with *Salmonella* at 11 MPN/g. Three isolates from the pie were serotyped and PFGE subtyped at CDC and were identified as *Salmonella* 4,5,12:i:- that was indistinguishable from the pot pie-associated outbreak isolate.

PRE-HARVEST FACTORS AFFECTING INTERNALIZATION OF ZOOONOTIC 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.

**SURVIVAL AND GROWTH OF ACID-ADAPTED AND UNADAPTED SALMONELLA
IN AND ON RAW TOMATOES AS AFFECTED BY STAGE OF RIPENESS AND STORAGE TEMPERATURE**
(L. R. Beuchat and D. A. Mann)

Several outbreaks of salmonellosis have been associated with the consumption of raw tomatoes. Once *Salmonella* attaches to the surface of tomatoes or infiltrates tissues, it can persist and may grow. Temperature and relative humidity affect the extent to which cells attach to ripe tomatoes. Populations of *Salmonella* Montevideo on the surface of mature green tomatoes stored at 10°C for 18 days have been observed to not change significantly. Populations of the same serotype inoculated on the surface of green tomatoes did not change significantly when tomatoes were treated with 100 ppm ethylene at 100% relative humidity and 20°C for 6 days. Several other *Salmonella* serotypes have been reported to persist on the surface of green as well as ripe (red) tomato fruits, leaves, and stems. Depending on temperature, relative humidity, and other factors, *Salmonella* may grow on the surface of tomatoes. *Salmonella* can also grow in diced red tomatoes at 22°C to populations exceeding 10^8 CFU/g. *Salmonellae* are known to be able to grow on sliced tomatoes.

Survival and growth characteristics of *Salmonella*, as affected by variety of tomato and stage of ripeness, has received little research attention. While Roma tomatoes have been reported to have a significantly higher pH than round tomatoes, survival of salmonellae in wounds and on the surface has been observed to be unaffected by variety. The behavior of acid-adapted *Salmonella* in and on tomatoes has likewise been given only meager research attention. Tolerance of *Salmonella* Baildon upon exposure to an agar medium at pH 4.5 is not influenced by the pH of tomato juice (4.8 or 5.8) or broth (pH 7.2) in which it had been grown. However, acid-adapted cells of *S. Montevideo* inoculated into homogenized Roma tomatoes are more resistant than unadapted cells to electron beam irradiation.

A study was done with the objective to determine if survival and growth of *Salmonella* in and on tomatoes is affected by the variety of tomato (round, Roma, and grape), stage of ripeness, and storage temperature. The influence of acid adaptation of cells and site of inoculation on survival and growth was studied. The influence of acid adaptation of cells and site of inoculation on survival and growth was studied. *Salmonella* grew in stem scar and pulp tissues of round, Roma, and grape tomatoes stored at 12 and 21°C but not at 4°C. Survival and growth was largely unaffected by variety and stage of ripeness at the time of inoculation. The pathogen did not grow on the skin of grape tomatoes stored at 4, 12, and 21°C. Survival and growth of *Salmonella* inoculated into stem scar and pulp tissues of round and Roma tomatoes were unaffected by prior exposure of cells to an acidic pH environment before inoculation. Results emphasize the importance of preventing contamination of tomatoes with *Salmonella* at all stages of ripeness, regardless of variety or previous exposure of cells to an acidic environment.

INACTIVATION OF PATHOGENS IN COMPOST MIXTURES AS INFLUENCED BY TYPE OF MANURE
(M.C. Erickson, C. Smith, X. Jiang, and M.P. Doyle)

During aerobic composting, heat is generated from the metabolic activity of thermophilic microorganisms and may contribute to inactivation of contaminant pathogens at internal sites of static piles. At the surface of compost piles, however, heat dissipation contributes to reduced temperatures and in turn reduced pathogen inactivation. It was the objective of this study to investigate whether pathogen inactivation at the surface would be affected by the compost composition and in particular the type of manure.

Chicken, cow, and hog manures served as the source of nitrogen in compost mixtures while straw and cottonseed meal were used as carbon amendments. Mixtures varied in the C:N ratio, having initial values of 20:1, 30:1, or 40:1 and were inoculated with both gfp-labeled *Salmonella* spp. and gfp-labeled *Listeria monocytogenes*. Mixtures were placed in trays (simulating surface sites of static compost piles) and held in environmental controlled chambers at 20° or 30°C and under different levels of light exposure. On a weekly basis, moisture levels in samples were adjusted to initial values (30% or 60%). Samples were periodically taken for enumeration of pathogens and measurement of moisture and pH.

At both 20° and 30°C, pathogen survival was greatest in compost mixtures formulated with cow manure followed by mixtures formulated with chicken manure and then hog manure. Regardless of the manure used in the compost mixture formulation, however, *L. monocytogenes* populations decreased faster than *Salmonella* spp. populations. Exposure to conditions simulating bright sunlight accelerated pathogen inactivation.

COMPETITIVE INHIBITION MICROORGANISMS FOR THE CONTROL OF ZOOBOTIC 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*.

THERMAL INACTIVATION OF SALMONELLA IN PEANUT BUTTER (L. Ma, G. Zhang, V. Mantripragada, P. Gerner-Smidt, and M. P. Doyle)

A large multistate foodborne outbreak caused by *Salmonella* Tennessee in peanut butter was reported in 2006. The objective of this study was to determine in peanut butter the rates of thermal inactivation at different temperatures of three *S. Tennessee* strains associated with the outbreak, in comparison to strains of *Salmonella* of other serotypes (Enteritidis, Typhimurium, and Heidelberg). Commercial peanut butter was inoculated with *Salmonella* strains and heated at 71, 77, 83, and 90°C. At least three independent trials were conducted at each temperature and for each group of *Salmonella*. The thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (20 min) followed by slower death rates for the remaining cells. The nonlinear Weibull model was used to fit the curves and describe the thermal inactivation of *Salmonella* in the peanut butter. The calculated minimum times needed to obtain a 5-log reduction at all temperatures for the composited three outbreak-associated strains were significantly higher ($p < 0.05$) compared with those of the 5-strain mixture of other *Salmonella* serotypes. Forty-six min were needed to reduce the 3-strain mixture of *S. Tennessee* by 5-log whereas 38 min were needed for the 5-strain mixture of other *Salmonella* serotypes. These results indicate that the outbreak-associated *Salmonella* strains were more thermal tolerant than the other serotypes tested. Thermal treatments of peanut butter at 90°C for less than 20 min are not sufficient to kill large populations of *Salmonella* in highly contaminated peanut butter.